Review Article

Prospective therapeutic strategies of microbiome engineering and human metagenome manipulation: A mini review

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Abstract

Gut microbiota is a complex ecosystem with a unique diversity of microorganisms living in the digestive tracts of humans and animals and has a strong impact on human health. This microbial relationship plays a crucial role in the metabolic health of the human host. Any dysbiosis in this relationship causes various metabolic, immunological, neoplastic, and functional diseases. For the proper maintenance of relationships between gut microbiota, health, and disease, a few strategies including the use of probiotics/prebiotics, antibiotics, fecal microbial transplant, and drug targeting have been used. Gut microbiota management strategies in balance with dietary modifications signify an interesting field of research, however, associated data along with a deeper understanding is mandatory for the improvement of more targeted and specified therapeutic strategies. In this review, we will address the exciting potential, emerging challenges, and current directions in human microbiome engineering strategies. Additionally, this review analyzes the significance and use of microbiome engineering for the improvement of human health.

Keywords: Antibiotics; Diet; Gut microbiota; Prebiotics; Probiotics; Microorganisms

Introduction

Microorganisms existing in the digestive tract of humans, animals, and insects form a complex community, and the collective aggregate of their genome forms the gut metagenome [1]. Human microbiota resides in human tissues, bio-fluids, oral mucosa, saliva, gastrointestinal (GI) tracts, skin, mammary glands, uterus, placenta, seminal fluid, lungs, etc. [2]. Among microbial groups, the gut flora is regarded as most important to the host as ~100 trillion (10^{14}) microorganisms residing in the GI tract hold

150 times as many genes as the human genome. There is a strong symbiotic relationship between gut microbes and humans in which a continuous source of nutrition is supplied by humans; in return, gut microbes provide various health benefits [3]. It has been estimated that the human microbiome is composed of more than 5000 strains of microbes and more than 1000 types of microflora. Principally this environment is dominated by anaerobic bacteria however, others include viruses, protozoans, archaea, and fungi. The microbiome is primarily

defined by two bacterial phylotypes; *Bacteroidetes* and *Firmicutes*, whereas a relatively small amount of *Actinomyces*, *Proteobacteria*, *Fusobacterium*, and *Verrucomicrobia* also exist. The composition of gut flora varies with human development and is affected by several factors including host genetics, age, nutrition, antibiotic usage, and mode of birth. Babies receive the initial microbiome from their mothers and after 1 year; the newborns form their own complex gut microbiome like adults [4]. There is a strong balance in the enteric microbiota that deliberates health assistance and contributes to maintaining the homeostasis further ensuring human health and safety [5]. Gut flora helps us in proper food digestion, activation of certain drugs, production of short-chain fatty acids (SCFAs) that help in the modulation of gene expression, generation of molecules that decrease inflammation, and plays a vital role in the initiation, growth, training, and functioning of the immune system. Conversely, dysbacteriosis of healthy gut microbiota results in the drop of this mutualistic association and causes many ailments like obesity, metabolic syndrome, diabetes (Type I, II), inflammatory bowel disease (IBD), irritable bowel syndrome, colorectal cancer, celiac disease, and a wide range of mental and neurological disorders [6, 7]. Thus, there is a strong need to comprehend the microbiome and advance understandings into elements that further add to differences in microbiomes. In this review, we have analyzed the significance and use of microbiome engineering, predominantly for the improvement of human health, and exploring the challenges and forthcoming human microbiome engineering strategies.

Dietary effects on gut microbiota

Diet is one of the major factors driving the configuration, conformation, and metabolism of the gut microbiota. The total sum, variety, and equilibrium of the key dietary

macronutrients (proteins, carbohydrates, and fats) have an excessive influence on the large intestinal microbiota [8]. Since the 1960s, there is a heated debate among the scientific community on the prospects that diet may affect the gut microbiota. Experiments have shown that when germ-free mice were subjected to a high-fat and high-sugar western diet, changes occurred in the structure of the intestinal microbial community with increased numbers of members of the phylum *Firmicutes* and decreased profusion of phylum *Bacteroidetes* [9]. Also, intestinal microbiota can change and remodel its composition swiftly by deviation in the diet for a short time. To observe the differences and reproducibility in gut microbiota, animal and plant-based diets were given to 10 American volunteers (between the age of 21 and 33). During animal-based diet consumption, the richness of bile tolerant microorganisms such as *Alistipes*, *Bilophila*, and *Bacteroides* increased, whereas a decrease was noted in the profusion of *Firmicutes* involved in processing nutritive plant polysaccharides (*Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*). On the other hand, short-term dietary forms persisted ineffectually in producing major variations in ‘enterotypes’, that are distinct as three groups of genera subjugated by *Bacteroides*, *Prevotella*, and *Ruminococcus* [10]. Thus, the ability of human gut microflora to rapidly change their composition and functionalities can be directly associated with the evolution of human beings with respect to the unavailability of some food sources over time. In past, humans were dependent on the attainment of hunting for animal-based diets, while additional plant-based nutrient consumption was more common, so these quick variations in nutrition improved the selective pressure for increasing the flexibility of functional components of the gut microbiota [11, 12]. Recently, mouse

model studies suggested that due to modern animal-based diet patterns, the bile acid biosynthesis mechanism is facing critical alterations in which high production of bile acid known as deoxycholic acid occurs. Consequently, it results in stimulating liver cancer. It has also been observed that high fat/ animal-based diets may promote the growth and development of *Bilophila wadsworthia* (a gram-negative sulphite-reducing bacteria), which is one of the signals for triggering IBD. In another clinical study, when a fat-rich and western-style diet was given to healthy individuals, increased plasma endotoxin levels were observed in comparison with individuals who were on an isocaloric steady diet. This can be a result of a disturbance in the gut microbiota [13]. On the other hand, plant-derived diets have a high fiber content than animal-derived diets. Thus, bacterial fermentation of nutritional polysaccharides can deliver 10% of the energy consumption which can stimulate deviations in the gut microbiota to perform numerous favorable functions including defense from inflammation, cardiac diseases, obesity, diabetes, and high blood pressure [10]. A comparative study [14] showed the effect of diet on host physiology by converting the plant polysaccharides-rich diet (low-fat) to an animal based, high-sugar diet (high-fat) in germ-free mice colonized with a human gut microflora. Results confirmed that due to dietary changes, gene expression patterns and metabolic pathways of the microbiome changed within a day. Diet having fiber enrichment caused plenty of *Bacteroidetes* and decreased the richness of *Firmicutes* [15].

Various nutrients like vitamins, fats, amino acids, or nutritional fiber consumed by the host are assimilated and transformed into supplementary metabolites by intestinal microflora. Certain yields of these biochemical reactions, such as SCFAs, biogenic amines or additional amino-acid-

derived metabolites such as serotonin (a neurotransmitter) or gamma-aminobutyric acid (GABA), could be naturally active in health and disease conditions. Production of such complexes also induces variations in microbial composition. Due to the fermentation of dietary non-digestible carbohydrates in the intestinal lumen, SCFAs such as acetate, propionate and butyrate can be produced. These metabolically active SCFAs are involved in various biological mechanisms providing metabolic energy bases for human colonic epithelial cells (Fig. 1).

Currently, the main emphasis of microbiome understanding is primarily on the management and preclusion of dysbacteriosis (dysbiosis) and related conditions. Due to lack of complete knowledge, it has not been utilized to its complete perspective and huge section of human microbiota is still to be discovered. However, it is expected that in near future, the finding of leading and exceptional members of microbial groups serving explicit constructive functions is possible to link this breach and might become the backbone of bacteriotherapy for numerous disorders. Microbiome engineering will not only broaden this possibility to meet pre-emptive and therapeutic but also the investigative prerequisites [16].

Most of the major diseases including obesity, colon cancer, diabetes, and inflammatory bowel disease have all been associated with fluctuations in the composition of the human gut microbiome. For the proper understanding, detection, and therapeutic treatment of gut dysbiosis, we require more cultured and refined tackles like microbiome engineering and transplantation. To control the configuration and functional aspects of the microbiome, great efforts have been made including the introduction of new members to the community by using probiotics and fecal transplantation, such as elimination of

unwanted members by using antibiotics and intestinal lavage methodologies. Still, these approaches are nonspecific and frequently result in unpredictable and random endings [17]. Recently, many therapeutic and targeted

therapies have been employed to get more specific results. We will address the major ones including prebiotics, probiotics, dietary emulsifiers, fecal microbial transplants, antibiotics, and synthetic microbes.

Figure 1. Intestinal microbial communities cause changes in human health status and different disease states.

Restoring microbiota through prebiotics, probiotics, and dietary emulsifiers

Non-viable dietary compounds (usually non-digestible fiber compounds) such as insulin, oligofructose, and gluco-oligosaccharides that deliberate health benefits on the host accompanying with modulation of microbiota are known as prebiotics [18]. Research shows that administration of prebiotics results in improved production of SCFAs that act as a as nutrition for colonic walls, and moderation of ulcerative colitis indications thus beneficial to inflammatory bowel disease or Crohn's disease [19, 20]. Fermentable oligosaccharides (e.g., fructans)

also called fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), are poorly absorbed materials. They have been shown to affect irritable bowel syndrome symptoms thus, restriction of foods containing FODMAPs have been sightseen as a prospective therapeutic intervention [21].

Probiotics are microbes (bacteria or yeast) that provide health aids when consumed and are called as good or helpful bacteria as they keep the gut healthy. The mechanism of action of probiotics is; if the body loses good bacteria or dysbiosis occurs, they help to replace them effectively further treating a

wide range of gastrointestinal diseases. Most of the recommended probiotics come from two groups: *Lactobacillus* and *Bifidobacterium animalis* [22]. Recent studies have shown that probiotic bacteria adjust the settlement and eradication of pathogens in the gut, and modulate the mucosal immune system [23, 24]. Probiotics are regarded as a significant prophylactic or therapeutic approach for several mucosal and non-mucosal immune-related disorders, such as IBDs, celiac disease, metabolic syndrome, and diabetes [25]. Probiotics have been fruitfully employed for the treatment of antibiotic-associated diarrhea and have antimicrobial, antimutagenic, anti-diarrheal, and anticarcinogenic properties. They have the potential to show progress in lactose metabolism, drop of serum cholesterol levels, immune system stimulation, and decrease of *Helicobacter pylori* infection [26]. The clinical effectiveness and ability of probiotics and prebiotics have been demonstrated in several clinical settings thus blend of prebiotics with probiotic bacteria has the potential to change gastrointestinal flora for the gain of thoroughgoing health benefits [27].

Dietary emulsifiers cause intestinal inflammation, metabolic syndrome, and inflammatory bowel diseases; apart from that, they are also involved in the prevalence rate of these disorders. Carboxymethylcellulose and polysorbate 80 are two synthetic emulsifiers that affect the intestinal microbiota and are known to cause bacterial translocation across the intestinal epithelium. The dietary emulsifiers tested in a mini bioreactor arrays model proved adverse effects on human intestinal microbiota [28]. Some food additives, too, are being used frequently but their impacts on human microbiota are still to be investigated. It is further indicated that clinical trials are required to test whether emulsifying agents have little or no effect on microbiota, so that,

the usage of detrimental compounds might be reduced.

Fecal microbiota transplant (FMT)

The technique used for the restoration of gut microbiota back to their normal density and diversity to attain proper gut functionality is called fecal microbiota transplant or stool transplant [29]. In this process, beneficial intestinal bacterial and yeast strains are taken from a healthy donor and implanted into the colon of a person who is missing the essential gut microflora due to having a functionally improper digestive system. FMT may prove effective and an operative strategy for the treatment of IBD and *Clostridium difficile* infection for which some early trials have shown success. A case study has shown the cure rate of 80-90% via FMT with no stern adverse procedures in the short term. The four main types of fecal transplants used in human medicine are mentioned in (Table 1) [30].

In the last 5 years, FMT has gained the consideration of physicians, consultants, and the US Food and Drug Administration (FDA). Fecal matter, if used to modify the physiology of a receiver, meets the federal definition of a drug, and consequently falls under the FDA's monitoring remit [31, 32]. FMT has been tested in the treatment and management of many diseases, including metabolic disorders, autoimmune and hypersensitive diseases, neurodevelopmental and neurodegenerative conditions, prolonged fatigue syndrome, etc. Recent studies have shown that microbiomes of super-donors are greatly effective in fecal microbiota transplant. It was observed that administration of FMT capsules from multiple donors resulted in continuous alterations in the structure and function of the gut microbiome. Promising results have been shown in many reports, though they are dealing with a very little number of patients and supplementary well-designed, randomized well-ordered trials are

compulsory for the establishment of the effectiveness of FMT for these ailments. Additionally, FMT studies have an inherent

limitation due to the variability of individual's stool composition [30, 33, 34].

Table 1. Types of fecal transplantation

Type	Requirements	Advantages/ Drawbacks
Single donor fecal transplantation	Single recipient is required (donor should be close relative or friend). Donor should be tested for the absence of pathogenic bacteria and particular diseases. Donor must be tested for the absence of pathogens and specific diseases.	Long delayed and often costly.
Multiple donors' fecal transplantation	Donor should be tested for the absence of pathogenic bacteria and particular diseases. Stool banks should be established.	No published microbiome and metabolomic data till yet.
Autologous feces transplantation	Collection of fecal samples before therapeutic involvements and proper storage.	No published microbiome and metabolomic data till yet.
Anaerobically cultivated fecal transplantation	Donor's selection and pathogen screening is only necessary once.	Best and cheapest method

Administration of antibiotics

Many strategies can be employed to alter the human gut microbiome. Animal model studies have shown that various antimicrobial peptides, like *thuricin Clostridium difficile* (thuricin CD) and *pyocin S5* were used for narrow spectrum targeting of pathogenic bacteria. This resulted in the reduction of the chance of resistance and disturbance to the gut microbiota [35]. The results provided the possibility of developing a very effective targeted methodology for removing *C. difficile* in the colon, without collateral impairment [36]. It has been observed that the composition, taxonomic richness, diversity, and evenness of gut microbiota can be altered by the administration of broad-spectrum antibiotics. Antibiotics cannot only change the composition of taxa but also affect the expression level of genes, protein activity, and whole metabolism of the gut microbiota. *Ex vivo* incubations of fecal samples with various antibiotics have shown an increased percentage of gut microbiota cells with damaged and impaired membranes. This means that there is a change

in the active populations of the microbiota, antibiotic resistance genes, stress response and amplified expression in phage induction [37, 38]. However, one of the major threats of excessively widespread use of antibiotics is increased resistance of bacterial pathogens to antibiotics that is becoming a global challenge. The (Table 2) shows that how a simple antibiotic course will cause turbulence in the composition of gut microbiota [39].

Synthetic microbes as drug delivery systems

Studies have shown that some diseases of the GI tract could be treated by orally administering a synthetic bacterium that can crisscross to the preferred location, engraft, and start supplying a drug. From engineered microbes, the recombinant expression of therapeutic biomolecules can help resolve an infection, stop inflammation, and treat metabolic conditions. This method is useful for protein containing compounds as they cannot pass through an acidic stomach environment in addition to the cost-effective method [17, 47]. It has been reported that genetically engineered *Lactobacillus jensenii*

stopped the spread of chimeric simian/human immunodeficiency virus (SHIV) in a rhesus macaque monkey model. *Lactobacillus jensenii* was engineered to express an antiviral molecule (cyanovirin-N) and it not only reduced the incidence of SHIV but also the ultimate viral contents when directed as a prophylactic treatment [31, 48].

Metabolic diseases like obesity and diabetes are also being addressed successfully by drug delivery systems. A previous study reported that obesity was reduced in mice by the introduction of genetically engineered *E. coli* expressing therapeutic factors in gut microbiota. It was shown that administration of engineered NAPE-expressing *E. coli* Nissle 1917 bacteria in drinking water for 8 weeks successfully resulted in reduced levels of obesity even fed with a high-fat diet [49].

Another condition known as hyperammonemia (in which an excessive amount of ammonia gathers systemically thus causing neurotoxicity and encephalopathy in individuals having a liver disease) can be recovered through microbial engineering. In mouse model studies, it has been observed that community-wide urea metabolism can be altered by using genetically modified gut microflora. In these studies, the existing endogenous microbiota were depleted out and a well-defined microbial community displaying low urease activity were transplanted which resulted in continuously stable urease levels [31]. The (Table 3) shows detailed results of microbiome engineering for different diseases, as modified from [16].

Table 2. Summary of research on the influence of antibiotics on the human intestinal microbiota

Antibiotic regime	Subjects	Short term results	Ref.
		long term results	
5 days orally Amoxicillin	6 (adult healthy volunteers)	After 24 hours, alteration in dominant species occurred. After 4 days: 74% average similarity as compared to pre-treatment.	[40]
		88-89% similarity in 1-2 months.	
Amoxicillin	28 (young pediatric patients)	Decreased number of <i>Bifidobacteria</i> and <i>B. fragilis</i> species compared to non-treated kids.	[41]
		No changes reported	
Orally 7 days Clindamycin	4 healthy adults/ 4 controls	Day 7, 21: large and consistent shift in composition.	[42]
		3, 6, 9, 12, 18 and 24 months: large and constant shift in composition	
Orally 5 days Ciprofloxacin	3 healthy adults	In the gut affected ones, abundance of the bacterial taxa; reduced taxonomic richness, multiplicity, and uniformity of the community.	[43]
		1 and 6 months: productivity, diversity, and constancy comparable to pre-antibiotic state, certain long-term losses.	
Metronidazole + Orally 7 days clarithromycin	3 Healthy adults + 3 controls	Intense reduction in diversity, specifically loss of <i>Actinobacteria</i> , in both throat and feces.	[44]
		1 year: diversity levels recovered to pretreatment states	
Ampicillin and gentamicin (within 48 h of	9 pediatric Patients + 9 untreated infants	4 weeks (after treatment): antibiotic-treated newborns had greater amounts of <i>Proteobacteria</i> and lower amounts of <i>Actinobacteria</i> along with genus <i>Lactobacillus</i> .	[45]
		2 months: Higher levels of <i>Proteobacteria</i> but recovered levels of <i>Actinobacteria</i> and <i>Lactobacillus</i> .	

Birth)			
Oral administration of broad-spectrum antibiotics	21 adult patients	25% reduced microbial diversity was observed by treatment with Fluoroquinolones and b-lactams; Increased percentage of Bacteroidetes taxa; average microbial load was increased roughly two-fold by B-lactams.	[46]
		None reported	

Table 3. Engineered microflora

Engineered commensal microflora	Induced modification and result	Ref.
<i>Bifidobacterium longum</i>	In mice, recombinant <i>Salmonella</i> flagellin's expression on the cell surface provided defense against infection from <i>Salmonella typhimurium</i> .	[50]
<i>Lactobacillus casei</i>	In mice, expression of protein C (<i>pneumococci</i>) resulted in its reduced nasopharyngeal colonization.	[51]
<i>Lactobacillus</i>	Oral administration of engineered human commensal bacteria engineered improved hyperglycemic condition in diabetic mouse models by reprogramming intestine cells into glucose-responsive insulin-secreting cells.	[52]
<i>Lactobacillus jensenii</i>	In monkeys, colonization of vaginal mucosal cells and secretion of cyanovirin-N provided defense against simian human immunodeficiency virus (HIV) infection.	[53]
<i>Caulobacter crescentus</i>	Display of anti-HIV antibodies resulted in neutralization of HIV.	[54]
<i>L. jensenii</i>	Anti-HIV; chemokine [Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES)] and C1C5 (RANTES) expression resulted in the reticence of HIV infection in CD4 ⁺ T cells and phagocytic cells.	[55]
<i>E. coli</i>	Production of AI-2 (autoinducer-2) and CAI-1 (cholera autoinducer 1) in high concentration resulted in suppression of cholera toxin assembly by <i>V. cholerae</i> .	[56]
	Engineered <i>E. coli</i> commensals bounded precisely to the HSPGs (heparan sulphate proteoglycan) on colorectal cancer cells and secreted the myrosinase and transformed host-ingested glucosinolates, thus engineered microbes attached with glucosinolates resulted in more than 95% inhibition of murine, human and colorectal adenocarcinoma cell and cell line proliferation in vitro.	[57]
	Chimeric cell wall lipoglycans copying GM 1 ganglioside finally deactivated cholera toxin and heat-labile enterotoxin of <i>E. coli</i>	[58, 59]
<i>Lactobacillus paracasei</i>	Expression of adhesion protein of recombinant <i>Listeria</i> resulted in competitive inhibition of <i>Listeria monocytogenes</i> to bind with its receptor.	[60]
<i>Lactococcus lactis</i>	Expression of surface-associated flagellin of <i>Bacillus cereus</i> CH strain resulted in competitive inhibition of adhesion of intestinal pathogens like <i>Salmonella enterica</i> .	[61]

Future perspective

Evidence recommends that treatment with probiotics, prebiotics, antibiotics, dietary

modification, FMT or drug targeting would expressively alter the comparative amount of the gut bacterial species and can result in

the improvement of several metabolic and neurological diseases. Besides this, an adaptation of the GI microbiota may have intense metabolic penalties and concerns too. Whatsoever the method used, alteration and reformation of the gut microbiota is risky thus limitations are always there in our capability to freely examine the intestinal microbial setting *in situ*. There is a strong need and gap in our understanding of the interaction between gut microbiota and human health in the normal and diseased state. Only then, any effective strategy separately or combined with other methodologies can be applied to cure the disease. As up-to-date molecular methods like sequencing tools are approving the identification of various microbial species present in human GI tracts, so it's an opening to identify and categorize the types of genes that they keep. Enhanced consideration of how these genes respond to bacterial signals that affect human physiology and precise editing technologies will provide open access to the development and improvement of new treatment models. Sensibly targeted antibiotics and microbiome editing technology have the potential to remove or suppress the selected constituents and mechanisms of the human microbiome further encouraging the proliferation of advantageous microbes to exploit sustainable fluctuations in the human microbiome. Though it is evident that enormous potential subsists for manipulation of the human gut microbiota for therapeutic consequence, it is manifested that a combination of scientific methodologies, new tools, and approaches with more investigation and exploration are desired to realistically target microbe-directed treatments, prevention, and management conferring to the disease state.

Author's contributions

Contributions in writing the main draft of the manuscript: M Asghar & J Khan,

Contributed to editing and proofreading the manuscript: H Khan & R Bibi.

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