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## HEALTH AND ENVIRONMENTAL APPLICATIONS OF GUT MICROBIOME: A REVIEW

### ZDROWOTNE I ŚRODOWISKOWE ZASTOSOWANIE MIKROBIOTY JELITOWEJ: PRZEGLĄD LITERATURY

**Abstract:** Life on Earth harbours an unimaginable diversity of microbial communities. Among these, gut microbiome, the ecological communities of commensal, symbionts (bacteria and bacteriophages) are a unique assemblage of microbes. This microbial population of animal gut helps in performing organism's physiological processes to stay healthy and fit. The role of these microbial communities is immense. They continually maintain interrelation with the intestinal mucosa in a subtle equilibrium and help the gut for different functions ranging from metabolism to immunologic functions like upgradation of nutrient-poor diets, aid in digestion of recalcitrant food components, protection from pathogens, contribute to inter- and intra-specific communication, affecting the efficiency as disease vectors etc. The microbial diversity in the gut depends upon environmental competition between microbes, their sieving effects and subsequent elimination. Due to wide diversity of anatomy and physiology of the digestive tracts and food habits, the gut microbiome also differs broadly among animals. Stochastic factors through the history of colonization of the microbiome in a species and *in situ* evolution are likely to establish interspecies diversity. Moreover, the microbes offer enormous opportunity to discover novel species for therapeutic and/or biotechnological applications. In this manuscript, we review the available knowledge on gut microbiome, emphasising their role in health and health related applications in human.

**Keywords:** gut-microbiota, environment, evolution, Wolbachia, enterotype

## Introduction: Gut flora and environment

More than 100 years ago, based on his extensive studies on intestinal bacteria, Professor Arthur I Kendall portrayed the alimentary canal "*as a singularly perfect incubator*" where different levels of reactions take place through a range of bacterial population [1]. The alimentary canal or the gastrointestinal tract (GI tract) of human is ~5 m long (oroanal length), having average inner diameter of 2.5 cm and 4.8 cm at small intestine and large intestine respectively, with the average total surface area ~ 32 m<sup>2</sup> [2]. It is estimated that, around 60 tons of food passes through the tract during the average life of a human [3]. The

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gut is home to approximately 100 trillion bacteria, that outnumbers the total population of human somatic and germ cells tenfold and represents a combined microbial genome well in excess (around a hundred times of genes in aggregate) of the human genome [4]. This association benefits the host in digestion of food materials, subsequent absorption of macromolecules, fermentation of undigested carbohydrates and assimilation of short chain fatty acids, SCFA (butyrates, propionates, acetates etc.). Gut flora also help in synthesizing vitamins (vit-B, and vit-K), metabolising bile acids, sterols and other compounds like xenobiotics [5, 6]. Despite their important contributions, gut microflora may pose serious threat to health by exposing the GI tract to various dietary antigens, and their own metabolites (e.g. endotoxins, phenols, indoles, hydrogen sulphide, ammonia, etc.) produced as a result of fermentation of the substrates available in the gut [2, 5, 7, 8]. Collectively, this gut associated microflora having diverse physiological functions equal to a virtual organ within an organ, is popularly referred to as gut microbiome [9, 10].

Microbiome, the term originally coined by Joshua Lederberg, may be defined as “the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space” [11]. Later, Ley et al. [12] described microbiome as the “complete collection of genes contained in the genomes of microbes living in a given environment (i.e. the set of genes contained in a microbiota)”. It is basically the combined genomes of the microbial communities that reside in specific areas of the body like skin, gut, etc. Accordingly, gut microbiota or gut flora is the diverse assemblage of microorganisms living in the digestive tracts. It is an active and a diverse microbial ecosystem that closely interacts with host to provide immunity, digestion, assimilation, synthesis, metabolism of varied compounds, storage of fats, and prevention of growth of pathogenic bacteria, thus ensuring wellbeing of the host. Due to their diversified functions, some scientists have deemed them as the “forgotten organ” in our body [6, 10, 13]. Host-microbe interactions primarily occur along the surfaces of the gut mucosa, where, a bi-directional host-flora exchange takes place. Commensal bacteria usually compete and consume the available nutrients in ecological niches thereby reducing the burden of nutrient overproduction, which may in turn help in controlling pathogenic microbial competitors [14]. Depending upon the structural pattern, physiology, food materials and feeding habits, huge variations in gut microbial community is found in different species. In human, up to 60% of the dry mass of faecal matter contains bacteria as they make up most of the flora in the gut [15]. In the distinct habitats of the gut, dynamic microbial diversity may be established in the course of environmental competition & sieving effect and subsequent elimination between microbes. In addition to the microbial ecosystem within the body, scientists have quite recently unravelled that a diverse and important group of viruses (including phage viruses) also share different niches within the same ecosystem [16]. Amongst its vast subject area, this review tried to incorporate major aspects like the diversity, important functions and evolutionary aspects of gut microbiota. Apart from the human microbiota, gut microbiota of the insects have been extensively studied due to the ease of experimentation. Such studies have led to our understanding of the functional and evolutionary significance of gut microbiota.

## Diversity and role of insect gut microbes and their environmental implications

The insect's gut is represented by a large source of as yet unexplored microbial diversity that play important roles from utilization of different organic polymers, methanogenesis, nitrogen fixation to pheromone production, pesticide degradation and pathogen prevention [17-19]. Data on the different insect gut microbiome and their functional aspects is a vast area that require more scientific inputs [19]. The anatomy and physiology of the insect guts differ greatly and thus accommodate microbiota that varies in composition and number (as for example, the approximate number of gut bacteria ranges from 109 in honey bees, to 105 in a fruit fly, to negligible numbers in the plant sap-feeders) (downloaded from: <http://schaechter.asmblog.org/schaechter/2013/06/>). As for example, polyphagous Iberian geotrupid dung beetles *Thorectes lusitanicus* that feed on and digest wide array of diet like dung, acorns, fungi, fruits, and carrion harbours diverse group of gut bacteria that are ranging from aerobic, facultative anaerobic, and aerotolerant microbiota [20]. During metamorphosis of insect from larvae through pupae to adults, drastic changes in the gut microbiome can be noticed which is even dependent on the habitats. As for example, in mosquitoes, shift from aquatic to terrestrial habitats lead to dynamic changes in the gut microbial population, which is more or less constant in respect to the habitats it shares [21]. Till date, most of the gut microflora cannot be cultured and the diversity and population of the species vary from species to species, even in individuals of the same species.

Studies have found that the gut bacteria not only contribute to the nutritional aspects, but also play a role in enduring with the colonization of the gut by non-indigenous species, including pathogens, especially in the insects like termites and cockroaches those thrive on suboptimal diets. The transfer of plasmids and transconjugation between bacterial strains is a common phenomenon in this kind of insects suggesting that the gut is a "hot spot" for gene transfer. The relationships between the insect and its microbiome and its application are interesting areas for research [22].

A recent report on the gut microbiota of honey bee (*Apis mellifera*) again suggested that its gut flora play a critical role to govern important functions related to the health of host with 6 of 7 phylogenetic groups considered core to the honey bee hindgut [23]. In another experiment, where high levels of genetic diversity were found, Engel & Moran [24] suggested that the genetic variation and functional differences may be due to niche partitioning within this species that has emerged during evolution.

Insects are evolved with efficient systems for microbial recognition and signalling pathways to fight against variety of pathogens in the intestinal tract as the exposure of the host to the outside environment is most frequent [25]. As, for example, the gut of the fruit fly *Drosophila melanogaster* resembles the intestinal tract of human, which constantly in contact with diverse microbial pathogens as the fly feeds on rotten fruits, etc. In the response, these insects usually follow the defence through development of peitrophic matrix and epithelial integrity at gut level [25].

Similarly, some gut bacteria are reported to help the host insect to protect them from a range of pathogens. *Wolbachia*, a maternally inherited intracellular bacterium, found in approximately 40% of insects (and many arthropod species), are having the capacity to manoeuvring host reproduction that benefits infected females [26]. *Wolbachia* also provides pathogen protection of virus-infected *Drosophila* [27]. Consequently, different strains of

*Wolbachia* have also been shown to protect the host insect from bacteria, viruses, nematodes and the malaria parasite. It is also reported that when *Plasmodium gallinaceum*, a malaria parasite is stably transfected into *Aedes aegypti* mosquitoes, protection against the parasite is conferred [28, 29]. This broad-spectrum pathogen protection happens through immune priming that upregulates the basal immune response by inducing an array of reactions like cytoplasmic incompatibility (CI) in the presence of the symbiont and better preparing insects against subsequent infection by pathogens [30]. The similar kind of observation was found in transient infections established in the mosquito *Anopheles gambiae* [30, 31]. Biocontrol strategy for reducing dengue virus transmission to humans may be achieved in future through the *Wolbachia* symbiont-infected mosquitoes [25]. Bacteria like, *Wolbachia*, have an important role that modifies host fitness either by improving the ability of wild mosquitoes to survive pathogen infection or may alter the natural composition of gut flora that hinder the pathogen form colonization [30].

## Human gut microflora diversity in different conditions

The diversity of gut microflora in humans is enormous. Gut microbial communities signify a single source of diversity in respect to human genetics and metabolic activities and alteration of the population may also occur due to the changes in diet, age, lifestyle and disease conditions [32]. Longitudinal variation of the bacterial flora can be seen in the human gastrointestinal tract where the species level difference between proximal (oral regions) to distal (colonic region) is enormous with an increase in the bacterial content distally. The study showed that the oral cavity contains about 200 different species, the stomach is almost sterile, approximately 108 bacteria per g (dry weight) may be found in the ileac contents and up to 1012 bacteria per g (dry weight) are present in the colonic contents [33].

From 2009 onwards, through the advent of next generation sequencing and analysis, scientific community of the world came to know the existence of a number of species shared by all individuals (human) comprising the phylogenetic core of intestinal microbiota [34]. Apart from the genus *Bacteroides*, which is most abundant (constituting about 30%), genera like *Prevotella*, *Clostridium*, *Eubacterium*, *Peptococcus*, *Ruminococcus*, *Peptostreptococcus*, *Lactobacillus*, and *Bifidobacterium* play important roles in human intestine [35, 36]. The large intestine contains organisms belonging to over 30 identified genera and as many as 500 separate species or phenotypes. The major kinds of bacteria in the colon are obligate anaerobes, with the most abundant bacteria are associated with the genus *Bacteroides*, *Peptostreptococcus*, *Eubacterium*, and *Clostridium* [14, 37].

The range of human gut microbiota composition varies according to the age and geographic origins, pregnancy (changes resemble similar to metabolic syndromes such as diabetes, without any ill-effect) and its composition is considerably higher in adults than in children, while individualistic variation is more in children than in adults [38]. Further, individual microbiome composition in human strongly depends upon environmental exposures and diets which is having less &/or no genetic influence and even consistent in different populations [32]. With advent of knowledge in this vast field of research, scientist have recommended three dominant enterotypes, namely *Prevotella*, *Bacteroides* and *Ruminococcus*, that are not nation or continent specific and variation in intestinal microbiota is normally stratified, not continuous [39]. As for example, *Prevotella* is related with carbohydrates and simple sugars, while, *Bacteroides* enterotypes is connected with

digestion of animal proteins, amino acids and saturated fats: thus, one enterotype may dominate over other on the basis of diets. However, long term modulation of diets may lead to change of an individual's enterotype that also have medical implications related to health issues [39, 40]. In the study related to monozygotic and dizygotic twin pairs with similarity in obesity or leanness, it was found that a subset of common microbial genes, were shared among those twins [41]. As per the research reports, it is assumed that individuals share a “core microbiome” rather than a “core microbiota” [42].

## **Human microbes and the environment: Dynamic interactions**

Microbial colonization in the gastrointestinal tract of a human infant starts after its birth. An infant begins to acquire microbes during the birth process. Colonization of microbes have been seen within approximately twenty minutes after birth, in case of vaginally delivered individual, that mostly carry similar kind of microbiota of their mother's vagina: while infants delivered via Caesarean section harbour microbial communities typically found on human skin [43]. The attainment of gut microbiota keeps on changing over few years, however, within the first year of life, infants begins to resemble of microbiota that of an adult gut [44]. As the child grows, phylogenetic diversity of the microbes in infant gut considerably increases and enriched, but significant changes also happen during the onset of breast feed, introduction of cereal, development of fever and antibiotic treatment and beginning of cooked/formulated feed materials [42, 44]. The dynamic interaction between the human microbiota and the environment lead to invariable transfer of microbial communities between surfaces and individuals. Immense populations of viruses are present in the human gut and other body sites. The bacteriophages are also present in human gut in huge number (gut viromes): however, till date, their roles in human health are largely unknown [45, 46].

## **Role of the human gut microbe in health and immunity**

The digestion of varied compounds by host's gut microbiota is well known. Clustering of microbiome from different guts from human, together with carnivores, omnivores, and herbivores showed diet based differences in the microbial composition [41]. Significantly, the microbiome within the human gut permits the digestion of varied compounds via metabolic pathways that are not directly related in the mammalian genome, thus, greatly increasing the ability of humans to take out energy from diverse diets [41, 42]. The increased adiposity in individual is not only dependent on the quality or quantity of the diet, but the composition of the microbiome that can extract more energy and subsequent deposition from food [47]. The gut microbiota plays a critical role in obesity. As for example, in mice model, researchers have shown significant variation in the microbial composition within the obese mice with domination of Firmicutes group over Bacteroidetes group that can extract more energy from their diet [47]. However, microbe-induced obesity can be found in another mouse model, like TLR5 (Toll-like receptor 5) knockout mice, where, the altered microbiota by some means makes the mice hungrier, in comparison with their control counter parts [48]. In human as well, the modulation of gut microbiota might be helpful in therapeutic aspects for promoting weight loss in obese patients or for supporting weight gain in others [49]. Individual human microbiome is also get enriched with the microbes ingested with food. A special class of glycoside hydrolases used to digest porphyrin (a polysaccharide common in red algae) is also found in the human stool sample

as a gene in *Bacteroides plebeius*. However, this gene is not universally present across the different human race of the world but in Japanese individuals. Hehemann et al. [50] reported that in Japanese diet, seaweed is a common food item; the gene of which is also found included in the gut microbiome of individuals who prefers seaweed. Thus, microbes help greatly to increase the ability of the metabolism, and allowing us to digest and draw energy from a diverse range of food materials [42, 50].

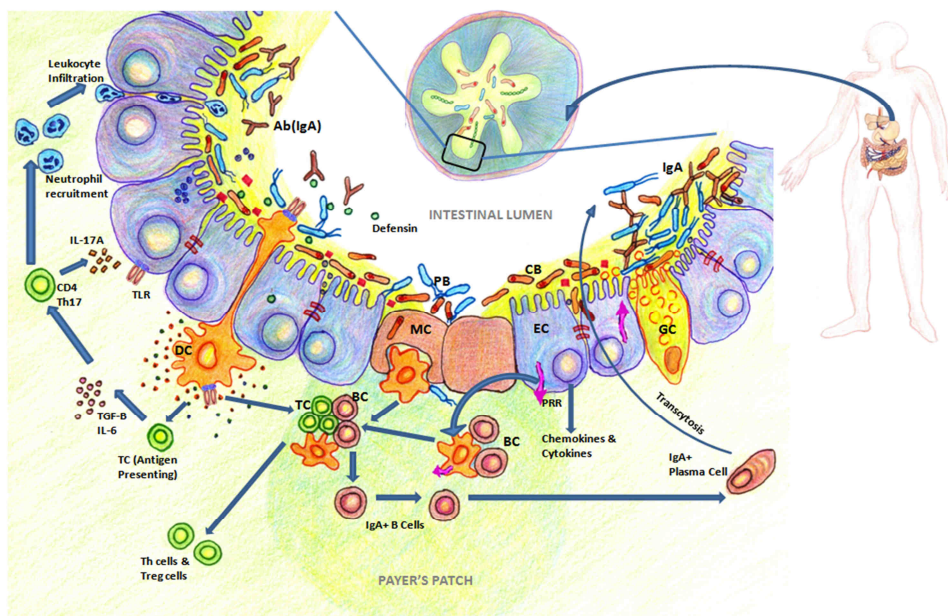


Fig. 1. Diagram showing gut-microbiota interactions in maintaining gut health through activation of immunological pathways. Ab - Antibody, CB - Commensal bacteria, CD - Cluster of differentiation, DC - Dendritic cells, EC - Epithelial cells, GC - Goblet cells, IL - Interleukin, MC - Mucous cells, PB - Pathogenic bacteria, PRR - Pattern recognition receptor, TC - T cells, BC - B cells, TGF- $\beta$  - Transforming growth factor beta, Th - T helper, TLR - Toll like receptor, Treg - T regulatory

The defensive function of the intestinal microenvironment formed by the microbes as a whole unit creates the barrier effect or resistance to pathogenic bacteria colonization. The gut bacteria play a key role on systemic immune systems of the host. The development of gut's mucosal immune system that starts during the infant stage of life continues to protect the gut in terms of physical components and function [51]. It is reported that microbial colonization of the gastrointestinal tract affects the composition of the gut-associated lymphoid tissue (GALT) [51]. Recognition of harmful bacteria within the gut and production of antibodies to pathogens by the lymphoid tissue associated with the gut mucosa is stimulated by the commensal bacterial community. The first flora settle in the infant gut are able to affect the immune response and subsequent responses throughout the life [44]. The expression pattern-recognition receptors (PRR) like toll-like receptors (TLRs) in the intestines is modulated by the gut bacteria that help the host the ability to discriminate between the pathogenic and commensal bacteria, activation of

immuno-sensory cells like, surface enterocytes, M cells and dendritic cells and to repair any damage due to injury [10, 14]. Nucleotide-binding oligomerization domain/caspase recruitment domain isoforms (NOD/CARD) are the other class of PRRs that recognize endogenous or microbial molecules or stress responses and forms oligomers that activate inflammatory caspases through activation of inflammatory cytokines and/or activate NF- $\kappa$ B signalling pathway to induce the production of inflammatory molecules [10]. Figure 1 depicts the role of human gut microbiota in maintaining gut health through activation of immunological pathways.

## Human innate gut microbiota and its variation

Antibiotics significantly affect the host's innate gut microbiota. Reports show that application of any broad-spectrum antibiotic (as for example, ciprofloxacin for 3-4 days) characterizes considerable variability in different individuals along with decrease in the taxonomic richness, diversity, and evenness [52]. Although, after approximately a week of treatment, gut microbial compositions began to retrieve, however, some taxa fail to return [52]. However, antibiotics are a recognized and effective mode of treatment for numerous infectious gastrointestinal conditions (e.g., infectious diarrhoea) and also beneficial to certain diseases like irritable bowel syndrome (IBS) and attenuating the effect of dysbiosis [53-55]. Promotion of community resilience is an interesting feature that helps the microbiota to overcome the stresses. Though the areas of microbiome plasticity have yet to be elucidated, some recent findings, especially fecal microbiota transplantation (FMT) has been successful in a number of studies [42, 56, 57].

Host microbiome can also be modulated to confer a health benefit to the host using 'probiotics' (live microorganisms that are not constituents of the host microbiome). Ringel et al. [58] showed that use of specific probiotic to some of the diseases associated with intestinal dysbiosis like antibiotic-associated diarrhoea, ulcerative colitis, pouchitis, and IBS-have yielded therapeutic benefits: however, the mode of the underlying effects yet to be known completely. A study on the consumption of a probiotic yogurt revealed the changes in expression of microbiome-encoded enzymes (related to carbohydrate metabolism), but no impact was found on microbial populations in faeces [59]. Several possibilities for the use of probiotics in dysbiosis conditions are there that may include use of a modified consortium or genetically engineered bacteria for targeted, enhanced benefits [59, 60].

Use of 'prebiotics' can also lead to change of gut microbiota that favours a more beneficial microbial community. Prebiotics are nondigestible food components that are selectively fermented by specific microbes in gut microbial community [61]. However, it is still not clear to the researchers if prebiotic involvement promotes constancy in the population or results only in temporary shifts of population that are beneficial [62]. Correspond a prebiotic with a probiotic (forming a synbiotic) may be a fresh approach to make dietary-induced changes in the gut microbiota [60].

## Role of gut commensal bacteria in partner selection

One of the important mechanisms for the origin of new species is mating preference through the selection of partner [63, 64]. The concept of speciation rests on the origin of isolating barriers that prevent actual or potential gene flow among populations and thereafter identification of the traits along with the evolutionary forces that control these

traits [65, 66]. Even though the gut microbiome manipulates several aspects of organismal fitness, its responsibility in animal evolution and the origin of new species is mostly unidentified. It has already been known that mating preferences in flies depend upon several factors like environmental conditions, such as humidity, temperature, pH of the rearing media, diet etc. [64, 67-69]. Dodd [68] primarily reported the positive assortative mating in *Drosophila pseudoobscura* that preferred mating partners reared for more than 25 generations in the same media: the flies reared on either starch-based or maltose-based media preferred mating partner came out from their own rearing media developing a population either of “starch flies” or “maltose flies”. Similar kind of study on *Drosophila melanogaster* was published by Sharon et al. [63] using molasses and starch as rearing media, where the mating preference of these flies appeared only after one generation and was maintained for at least 37 generations. Interestingly, mating preference was abolished after a treatment with antibiotic, signifying the important role of microbiota of fly gut for the phenomenon [64]. These observations triggered the scientific community to study further on the speciation and evolution of a population based on dietary substances. It is known that physical barriers like mountain ranges and rivers can separate closely related species and keep them away from cross-breeding. However, the microbes in an animal's guts could have the similar function in selection of mates. Further, gut microbiomes could have an effect on an organism's longevity and its capacity to reproduce. A study on termites *Zootermopsis angusticollis* and *Reticulitermes flavipes* treated with antibiotic showed the reduced diversity in their gut flora that led to reduction of beneficial microbes and subsequent malnourishment that led to the production of significantly less number of eggs [70].

In a recent study on the parasitic wasp *Nasonia* sp., Brucker & Bordenstein [71] showed that the bacteria in this insect gut of two newly diverged wasp species (*Nasonia giraulti* and *Nasonia vitripennis*) perform as a living barrier that prevents their evolutionary trail from mating together. The wasps delicately maintain a dissimilar sets of gut microbiomes, and after their cross-breed, the hybrids develop a distorted microbiome in the intestine that causes their premature deaths [71]. Further in this study, it was found that bacterial constituents and abundance are unequal in hybrids relative to the parental species and bacterium *Providencia* sp. dominates the parental species in the gut, while *Proteus mirabilis* in the hybrid one, which advocates that inter-breeding between two species brings about detrimental alteration to the gut flora, so the microbiome of *Nasonia* helps to remain the two species separate [71].

With the emerging concept of hologenome researchers are considering that the gut microbiome is vital to human evolution as well. Apart from other socio-psychological factors, human gut microbiome helps in adaptations like digestion, smell and the immune system that may have an effect on partner selection [72].

## Food, gut microbiota and evolution in human

About 4.4 to 2.5 million years ago, *Australopithecus* species marked the early evolution of our lineage that started as a split from prehistoric apes and was connected to a shift to food habits and climate change [73]. From *Australopithecus* through *Homo erectus*, modern *Homo sapiens* came about 1 million years ago when the climate of Earth became cooler, drier, and grasslands (C4-plant domination) expanded [12]. However, food might be the central machinery for human evolution. The comparisons between the



microbiomes of our evolutionary relatives have revealed genetic variability involved in the development of human-lineage specific traits. In the history of human ecology, starch played an essential role as the primary energy source, especially when evolution of agricultural based societies took place over the hunter-gatherers [12]. Gene responsible to produce starch hydrolytic enzyme salivary amylase (AMY1) not only duplicated in the human genome after the split of humans from the chimpanzee, but also, shows extensive variation in copy number, based on the diets, even in modern human [74]. Amazingly, on average, more AMY1 copies were found in individuals with high-starch diets (e.g., agricultural societies) than those with traditionally low-starch diets [12]. Another example is lactose tolerance in human which came after addition of milk in the diets is a very recent in our evolution. Introduction and fixation of this trait was found especially with populations coincides geographically with milk protein gene diversity in cattle [12, 75, 76].

Change in climate and related foraging behaviour and diet might help in evolution in human. The famous “grandmother hypothesis”, where the older females in a community started foraging and sharing plant roots, bulbs and tubers to their children, the behaviour that supported more pregnancies, post-menopausal longevity in adults, and better nourishment in children. The increased role of this bulb-tuber based diet is found consistent and the stable isotope composition was found similar to the tooth and bones of early human and African mole rat, a present-day consumer of such foods [12, 77, 78].

## Gut microbiota and health implications like cancer

Among the other human diseases, two most important global health problems are Diabetes and cancer. Recent data suggest that gut microbiota may have important implications in modulating the risk of these diseases and their therapeutics. Apart from genetic components, based on numerous clinical/epidemiological studies and experimental animal models it has been proposed that gut microbiota may contribute to the development of colorectal cancer (CRC) through exposure of the gut mucosa to the microflora and their metabolites [79]. Though such malignant transformations are poorly understood, but different mechanisms involving increased inflammation due to pro-inflammatory signalling, direct cytotoxic effects or carcinogenic metabolites produced by the microbes been proposed [80].

*Helicobacter pylori* (*H. pylori*) related gastric carcinoma is the widely-studied example of microbiota-induced GI tract carcinoma. Studies utilizing *H. pylori* and *H. felis* (murine model of *H. pylori* infection), have shown that the microbe may cause malignant transformations of the gut epithelium either through chronic inflammation of the gastric mucosa by induction of T cell-mediated cellular immune response [80-82], or by production of reactive nitrogen intermediates that damages DNA [83, 84]. Additionally, other bacterial populations have also been implicated in the development of colorectal carcinomas since it has been observed that gut microflora is altered in patients with polyposis stage or colon carcinoma, with increase in the diversity of *Clostridium* spp., *Bacteroides* and *Bifidobacterium* spp., as compared to *Lactobacillus* spp. and *Eubacterium aerofaciens* in subjects with low risk of CRC [85-87].

Apart from the direct involvement of gut associated microflora, epidemiological evidence implies that by-products of microbial metabolism of diet (e.g. hydrogen sulphide produced through metabolism of high protein diets by sulphate-reducing bacteria) might also lead to CRC [85, 88, 89]. It has also been reported that gut bacteria *Enterococcus*

*faecalis* may promote DNA damage in gut epithelial cells through production of extracellular reactive oxygen species (ROS) [90] or through DNA-damaging clastogens produced by *E. faecalis* activated macrophages, a phenomenon known as the bystander effect [91].

### **Fecal microbiota transplantation: Bacteriotherapy for future**

Fecal microbiota transplantation (FMT) or stool transplant or bacteriotherapy is the process of transplanting fecal bacteria from a healthy individual into a recipient [92]. Though this is a new branch in therapeutics, has been demonstrated to be an extremely effectual treatment for patients suffering from *Clostridium difficile* infection (CDI), which produces effects ranging from chronic diarrhoea to pseudomembranous colitis. Chronic diarrhoea is associated with an alteration of gut microbiota when a pathogen occupies the gut and in a number of conditions linked with intestinal mucosal damage or bowel dysfunction (as in inflammatory bowel disease, irritable bowel syndrome, or small bowel bacterial overgrowth) [57]. The emergence of hypervirulent strains of *C. difficile* in different parts of the world, especially in epidemic proportions in North America and Europe cause almost 110,000 deaths per year, where FMT has acquired growing importance with some experts calling for it to become first-line therapy for CDI [57, 93]. Further, faecal autologous bacteriotherapy (Autologous Restoration of Gastrointestinal Flora - ARGF) or FMT therapy using standardised filtrate composed virtually entirely of viable faecal bacteria in a colourless, odourless form is a promising branch that may help to address many diseases in future [94].

### **Recent studies on Human gut virome**

The human microbiome, especially the study of bacteria has remained a focus of research for more than a decade now. However, recently scientists have started understanding that bacteria aren't the only microorganisms that inhabit human bodies, but a diverse group of viruses also share different niches within the same ecosystem [16]. According to a rough estimate, there are almost 100 times more viruses in the human body, as compared to total number of eukaryotic cells. Viruses are omnipresent in nature: most of them were believed to be pathogenic, can infect every possible kind of living system [16, 95]. On the contrary, recent studies are suggesting that a wide diversity of viruses is part of normal human being and many of them may not be pathogenic, but help the individual to stay fit. These resident viruses are termed as 'human virome', and their identification through metavirome studies have opened new avenues in understanding highly complex human-bacterial-viral or human-viral interactions. Different viruses are now known to control the bacterial diversity present in different part of the human body [96, 97]. Additionally, thousands of endogenous viral remains are also known to be embossed in our genome, representing as much as 8% of our genome [98]. Among the metavirome studies, stool samples have been mostly explored to understand the human gut associated viral population, due to easiness in collection of adequate sample through non-invasive procedures [99].

Adult gut virome is known to harbour an abundant and varied community of both DNA and RNA viruses, majority of the DNA viruses being bacteriophages, while RNA viruses being plant viruses [100]. In one of the earliest studies on human gut virome (DNA viruses), approximately 1,200 different virus genotypes were recorded, majority of which

belonged to the *Siphoviridae* family of bacteriophages [101]. In another study focused on DNA virus diversity in a healthy 1-week-old infant, low diversity of viral community was observed, which was composed mainly of Caudovirales order of phages, and the viral community changed dramatically between 1 and 2 weeks of age [100]. Recent investigations utilizing extremely deep metagenomic sequencing have also confirmed the predominance of bacteriophages, affiliated to the families *Siphoviridae*, *Myoviridae*, *Podoviridae* and *Microviridae* in human faecal matters, some of which evolve at a rapid rate, as compared to the others, leading to great inter-individual diversity [45, 46, 102, 103]. An extensive review of different family of viruses detected in different parts of the human body has been published elsewhere [99]. It is now well agreed by the studies that phage communities in the human gut play a crucial role in the controlling pathogenic as well as non-pathogenic bacterial community structure and function. In addition to the control of bacterial population, bacteriophages normally studded in the mucus barrier (lining airways, intestine, oral cavity and other orifices) have been demonstrated to confer non-host derived immunity in human, suggesting a symbiotic relationship between phage and human [104]. Very interestingly, it has been observed that certain plant infecting RNA viruses, specifically Pepper mild mottle virus (PMMoV) is detected in significant amounts in human faecal matter [105]. Although plant viruses are believed to be non-pathogenic to humans, but a recent study on PMMoV has suggested the possibility of a pathogenic role of this plant viruses in humans [106]. Moreover, this and other studies have also suggested a dynamic and inter-individual difference in the human gut virome, depending largely on the diets, as has been demonstrated for gut bacteria [41, 106].

Nevertheless, our knowledge of gut associated bacteriophage community remains poor, since only a few well characterized gut-associated bacteriophage genomes are currently available, from which their possible contribution to gut ecosystem could be deduced. Remarkably complete genome analysis of the human gut-specific bacteriophage ( $\phi$ B124-14, infecting closely related gut-associated *Bacteroides fragilis* strains) revealed novel functions which were previously seldom associated with viral genomes or human gut viromes, and these functions could potentially be advantageous for both the phage and/or its host bacterium [107].

## Conclusions

Through the inheritance of varied processes, tools, and infrastructure after the completion of Human Genome Project, scientists started to delve into the fascinating field of gut microflora of the varied fauna. Evolution of all the eukaryotes occurred in the presence of diverse group of microbial communities that appeared as early as 3.25 billion years ago on planet Earth. The role of these microbial communities is immense. The gut microbes usually constitute the densest cell number in many species and have been found to be the most diverse in human. Newly branded the 'Human Microbiome Project' is now determining the most complex and abundant microbial community and their interactions that have impacts from individual to community level [108]. Published results have pointed out the importance of gut microbiota in human health and in diseased conditions. Similarly, few specific bacteria in the gut flora of insects may help to reduce the progression of pathogens &/or diseases: these bacteria may also help economically important insects to increase the immunity and disease resistance against infection in future. Further, several biotechnological applications may be possible by utilizing many new findings. High

resolution microbial community structures, composition, function, ecology and evolution are being studied utilizing innovative molecular-metagenomic-bioinformatics techniques. However, understanding of the gut microbiome needs to address number of predictor variables including study design, sample collection and methodologies. Century old question of entwine genomics and functions of symbionts with their host genome may also get illuminated. A concerted efforts and collaboration of scientists of varied fields including microbiologists, molecular biologists, medical practioners, clinicians, physicists, bioinformaticians, and statisticians is the need of the hour to get proper accomplishment in this field.

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