Microorganisms are present on almost all parts of the human body that come in contact with the external environment including the gut, skin, oral cavity, genitourinary tract, and airway.¹
Of these microorganisms, more than two-thirds are present in the gastrointestinal (GI) tract. Approximately 99% of gut microbes are bacterial species, and it is not surprising that most of the microbiota research has focused on these organisms. Major technological advances in molecular biology have greatly expanded our knowledge of the gut microbiota and highlighted the complexity of this system and its crucial role in health and disease pathogenesis. The gut “microbiome” is an area of intense research, but current understanding of its role in human disease is still preliminary and largely descriptive. This article will provide a practical view of the composition and function of human gut bacteria and their role in GI and liver diseases.

**Tools for Studying the Gut Microbiota**

In the past, the study of microbiota depended mainly on culture-based techniques. This limitation significantly underestimated the true diversity and complexity of the gut microbiota, as many bacterial species cannot be cultured by standard methods. The advent of advanced molecular techniques greatly accelerated developments in the field. These tools provide a large amount of information in a short period of time. They may involve detection of a small portion of the nucleic acid of an organism for identification of the species or the entire genome and its products for functional characterization. For example, analysis of 16s ribosomal RNA (16s rRNA) has been used extensively in identifying bacterial species. This RNA is small in size but has enough difference in its variable region to allow for differentiation between species, making it an extremely useful target. A similarity greater than 97% is required for bacteria to be grouped into same species.

Although 16S rRNA is helpful in the identification of bacteria, it provides no information on genetic structure and function—information critical for the study of how organisms affect human health and the various perturbations that occur in the disease state. The application of “omics” technology can be used to gather this data. The bacterial gene pool can be identified by complete sequencing of DNA (genomics). To determine which

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**Figure 1. The overlapping role of the gut microbiome, host, and environmental factors in the pathogenesis of IBD.**

Dysbiotic changes in the gut microbiome may be influenced by diet and other environmental factors and predispose to IBD. A small proportion of IBD patients have demonstrable genetic susceptibility factors. From Hold et al World J Gastroenterol. 2014 Feb 7; 20(5):1192-1210. IBD, inflammatory bowel disease.
genes are actually being expressed, mRNA expression profiling (transcriptomics) can be performed or protein products (proteomics) can be identified. The metabolites produced by bacteria can be measured using mass spectrometry and nuclear magnetic resonance spectroscopy (metabolomics). The data provided by "omics" technology may be enormous and present challenges in interpretation and analysis, but the hope is that they ultimately will provide key insights into disease pathogenesis.

Realizing the important but daunting task of characterizing gut microbiota, various consortia have been formed and tasked with assessing human microbiota in health and disease. In 2008, the US National Institutes of Health launched the Human Microbiome Project to study the microbiome in gut, oral cavity, vagina, skin, and respiratory tract. From the data obtained, a reference database was established in 2012. The MetaHit (Metagenomics of Human Intestinal Tract) project funded by the European Union was aimed at characterizing gut microbiota. Other consortia include the Canadian Microbiome Initiative, the Australian Jumpstart Human Microbiome Project, and the Microbiome Diversity Project in Korea. These developments and initiatives indicate that the scientific community realizes the importance of research in this field and bodes well for the future.

**Development and Composition of Gut Microbiota**

Colonization of the GI tract with microbes begins during birth and the complexity and numbers increase progressively. By about age 2 or 3 years, the microbial composition of the gut starts resembling that of an adult. The diversity and number of organisms increase until late adolescence, after which they remain stable throughout most of adulthood. Changes may appear again after the sixth decade of life.

Numerous factors determine the composition of gut microbiota during childhood. These include the mode of delivery, diet, exposure to antibiotics, genetics, and maternal microbiota. For example, the gut microbiota of infants delivered by cesarean or fed on formula differs in composition from that of children delivered vaginally or fed on breast milk.

The human body harbors roughly microorganisms—far more than the number of cells in the body. In the GI tract, about 99% of the microbes are bacteria; archaea, viruses, and eukaryotes constitute the rest. An individual may have an average of 500 to 1,000 bacterial species in his or her gut. The microbial DNA collectively encodes for more genes than their host.

With such complexity, comprehending the microbial composition and their metabolism presents researchers a significant challenge. Most of the bacteria are anaerobes; the remainder are facultative anaerobes and aerobes. At the phylum level, about 90% of gut bacteria in adults belong to *Firmicutes* and *Bacteroidetes*. Other phyla include *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Fusobacteria*, *Spirochaetes*, *Tenericutes*, and *Lentisphaera* in infants, *Proteobacteria* and *Actinobacteria* predominate.

Adult microbial composition is also affected by several factors including genetics, malnutrition, obesity, smoking, use of antibiotics, and diet. For example, a diet based on animal fats and proteins increases organisms like *Alistipes* that are tolerant to bile and decreases the concentration of organisms like *Roseburia* that metabolize plant carbohydrates.

It has been proposed that the microbiota of an individual can be classified into 1 of the 3 enterotypes based on the variations in levels of the genera *Bacteroides*, *Prevotella*, and *Ruminococcus*. Type 1 enterotype has a high level of *Bacteroides*, whereas types 2 and 3 are dominated by *Prevotella* and *Ruminococcus*, respectively. This classification appears to have a functional significance and does not seem to be influenced by age, body weight, or geographical location; however, long-term diet may have an impact.

The number and diversity of microbes varies along the length and cross-section of the GI tract. The stomach has 10 cells/g of luminal content and these increase to 10^4 cells in the jejunum and 10^7 cells in the ileum. The colon has the highest concentration of bacteria at 10^{11-12} cells/g. When present, *Helicobacter pylori* is the main species in stomach. *Bacilli* and *Streptococcaceae* are prevalent in the small bowel and *Bacteroidetes* and *Lachnospiraceae* are common in the colon. The dominant bacterial colonies on the epithelial surface of the intestinal tract also differ from those in the lumen. This has implications when interpreting results from studies based mainly on fecal microbiota because it may not give accurate information about the bacteria on epithelial surface.

**Function of Gut Microbiota**

The commensal bacteria in the human gut play several important roles in the maintenance of health and physiology. They affect host gene expression and metabolism and they are essential for the development of a healthy immune system in the gut and maintenance of the gut barrier. Germ-free animals have a defective immune system with underdeveloped lymphoid structures and reduced immunoglobulin A–producing plasma cells. The microbiota contribute to maintaining a state of tolerance in the GI tract by influencing host T cells, including regulatory T cells. Considering the large volume of bacteria in the gut, this tolerance to commensals is critical in preventing unwanted immune activation, which would be deleterious for the host. Gut microbes may also influence development of the enteric nervous system, as shown in animal studies.
Another important function of gut microbiota is the extraction of energy from undigested food products. Bacteria metabolize many of these products especially fibers to short-chain fatty acids (SCFAs), which include butyric, propionic, and acetic acids. SCFAs serve as the energy source for colonic epithelial cells and they also affect cell differentiation and immune functions. The efficient energy extraction also may have the unwanted effect of promoting weight gain. It has been demonstrated that gut microbiota play a role in the development of obesity. The bacteria also produce other metabolites from dietary contents like hydrogen sulfide, phenols, and mercaptans, which may have harmful effects. Commensal bacteria prevent colonization of intestine by pathogens. This happens through several mechanisms, including competing for space and nutrients and production of antimicrobial substances like bacteriocin, protease, and peroxidase. Antibiotics disturb the commensal community and can sometimes lead to overgrowth of pathogens and ill health, including diarrhea.

Other functions of gut microbiota include production of vitamins and folic acid, which can be used by the host. They may metabolize xenobiotic compounds like antibiotics, which may affect drug bioavailability. They also metabolize a fraction of bile salts in the intestine. Apart from their role in the development and function of GI tract, gut microbiota also have demonstrated an influence on the development of the cardiovascular, endocrine, and nervous systems, among others.

Gut Microbiota and GI and Liver Diseases
Disturbance in the composition (dysbiosis) and/or function of gut microbiota has been linked to several diseases. However, because of the complexity of the microbiota, pinpointing the role of individual species of bacteria in the pathogenesis of a particular disease is challenging. Although there has been rapid progress, the current understanding of microbiota and human disease remains preliminary. Also, the cause-and-effect relationship has yet to be established for most conditions, as it is unknown whether the altered microbiota is the cause or the consequence of a given disease state.

Inflammatory Bowel Disease
Gut microbiota is considered a key driver of inflammation leading to inflammatory bowel disease (IBD) in genetically predisposed individuals. Among conditions of the GI tract, IBD has received close attention from microbiota researchers. This is not surprising considering the prevalence of this disorder and the role of gut microbiota in its pathogenesis (Figure 1).

Numerous studies have shown that the composition of gut bacteria in patients with Crohn’s disease and ulcerative colitis differs from that in healthy people. There is reduction in Firmicutes and Bacteroides; Faecalibacterium prausnitzii, a firmicute, has anti-inflammatory properties and has been shown to be reduced in in patients with IBD, and Proteobacteria and Actinobacteria are increased. Adherent invasive Escherichia coli were found in increased numbers in ileal biopsy specimens in patients with Crohn’s disease.

Similarly, the diversity of species seen in patients with IBD is less than in healthy individuals. Dysbiosis may lead to an increase in antigen exposure of gut immune cells, reduction in production of beneficial compounds like SCFAs, increased oxidative stress, and altered metabolism of bile acid, all of which may promote inflammation although the detailed mechanisms remain to be delineated. Host genetic and environmental factors may be affecting these changes.

GI Cancer
Another important focus of microbiota research is its role in development of GI cancer, particularly colorectal cancer (CRC). The composition of gut microbiota in patients with CRC and adenomatous polyps was found to differ from that of healthy controls. The bacteria linked to the development of CRC include sulfate-reducing Fusobacterium nucleatum and Enterococcus faecalis. Most CRCs progress through stages of accumulating genetic changes. Microbial dysbiosis can lead to genetic mutations by producing genotoxic metabolites or promoting inflammation. Sulfate-reducing bacteria like Desulfovibrio and Desulfovirnovas produce hydrogen sulphide and E. faecalis produces reactive oxygen species, both of which appear toxic to epithelial cells.

Diet may be implicated in the development of CRC through its effects on bacterial metabolism. Protein-rich diets encourage formation of harmful products like nitrosamines and heterocyclic amines by bacteria, whereas fiber in the diet is a source of production of SCFAs, which have protective effects. Another important site of cancer in the GI tract is the stomach. The role of H. pylori in the development of gastric cancer is well established. In areas with a high prevalence of gastric cancer, eradication of H. pylori has been recommended to prevent development of gastric cancer.

Functional Bowel Disease
Functional disorders are perhaps the most common GI problems for which people seek medical attention. Dyspepsia and irritable bowel syndrome (IBS) are 2 important conditions in this group. Symptoms are thought to result from abnormal GI motility, increased visceral sensitivity, and abnormal processing of signals in the gut–brain axis. Gut microbiota may affect motility and visceral sensation by promoting inflammation and aggravating the enteric nervous system. There are no consistent observations with regard to actual changes in gut microbiota, but some studies have shown that populations of Firmicutes and Proteobacteria are
increased and \textit{Bifidobacteria} and \textit{Actinobacteria} are reduced in patients with IBS.\textsuperscript{50–52} Interventions like probiotics, antibiotics, and a low FODMAP (Fermentable Oligo-Di-Monosaccharides And Polyols) diet, which are used to treat IBS, likely are beneficial because of their ability to alter microbial composition.\textsuperscript{21,53,54}

\textbf{Liver Diseases}

Abnormal function of gut microbiota appears to be a significant factor in the cause and progression of several liver diseases.\textsuperscript{55} Nonalcoholic fatty liver disease (NAFLD) and alcohol-related liver disease (ALD) have been areas of special interest in this regard. Alteration of gut microbiota by diet, alcohol intake, and other environmental factors leads to increased production of proinflammatory substances, including lipopolysaccharide (LPS).\textsuperscript{55} Exogenous compounds such as alcohol and bacterial products also increase the permeability of the intestinal epithelial barrier. A shift of microbiota toward one with more toxin-producing potential and increased gut permeability may result in significant leakage of bacterial toxins like LPS from the gut into circulation. This leakage would result in activation of Kupffer cells, stellate cells, and hepatocytes and drive inflammation, with resultant tissue damage and, subsequently, fibrosis.

Overgrowth of bacteria in the small intestine has been reported in patients with nonalcoholic steatohepatitis and has been associated with raised level of proinflammatory in these patients.\textsuperscript{56} \textit{Bacteroides} are reduced in numbers in patients with NAFLD and ALD.\textsuperscript{57} In ALD, activators of innate immunity like \textit{Enterobacteriacea} and \textit{Proteobacteria} are increased.\textsuperscript{57}

Animal studies involving microbiota transfer have shown that dietary factors alone may be insufficient to cause NAFLD and require appropriate microbiota.\textsuperscript{58} An important factor for NAFLD is obesity, and microbes can influence development of obesity by increasing energy extraction from diet and promoting fat deposition.\textsuperscript{30}

Gut microbes have been linked to hepatic encephalopathy, viral hepatitis, progression of cirrhosis, and development of hepatocellular carcinoma.\textsuperscript{55} Microbial composition has been shown to be altered in patients with minimal and overt hepatic encephalopathy—partly explaining the beneficial effect of luminal antibiotics like rifaximin in treating hepatic encephalopathy. The gut microbiota in patients with cirrhosis shows an increased concentration of organisms normally found in the oral cavity,\textsuperscript{60} although whether this is an artifact or has a role in the progression of the disease is unknown. Overall, data on gut microbiota and liver disease are still emerging and although exciting, at this stage conclusions are elusive.

\textbf{Potential Therapies}

The expansion in the knowledge of gut microbiota also has provided avenues for developing potential therapies. Various methods of modifying gut bacteria include administration of probiotics, prebiotics, and antibiotics or by FMT.\textsuperscript{65,66} Examples include antibiotics for treatment of diarrhea-predominant IBS and hepatic encephalopathy, and FMT for treating \textit{C. difficile} infection.\textsuperscript{62} Preliminary reports suggest FMT can be successful in patients with ulcerative colitis and probiotics may maintain remission in this condition but the validity of these observations is uncertain.\textsuperscript{67,68} Animal studies have shown that antibiotics can improve metabolic abnormalities associated with obesity.\textsuperscript{69} However, until we develop a clear understanding of the specific microbiota involved in the disease process and their pathogenetic mechanisms, therapies will be largely empirical. It also is unlikely that all bacterial species can be classified strictly as either beneficial or harmful because they may have multiple roles. Targeting them for therapies may deprive the host of their beneficial roles and predispose to other diseases. A cautious approach therefore is needed.

\textbf{Conclusion}

The gut microbiome doubtless has a major influence on human health and disease. Disturbance of the normal homeostasis of the microbiome (dysbiosis) has been linked to many GI conditions such as IBD and IBS. The role of the microbiota in metabolic pathways leading to obesity, diabetes, cardiovascular disease, and cancer is perhaps the most exciting area of study and offers the biggest challenge and the greatest reward in terms of ultimately reducing the burden of disease in society. Understanding why dysbiosis occurs, how it leads to disease, and how to restore normal homeostasis are major areas of future research. Gastroenterologists are the natural custodians of this research field and it is imperative that we dedicate all our energy, skill, and resources to conquering these challenges.


