*Review*

# Human stomach microbiota: Effects on health and disease

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## Abstract

The gut microbiota is a complex ecological community, consisting of trillions of microbes which include bacteria, viruses, fungi and protozoa. The stomach was previously considered as a sterile site uninhabited by microbes due to its hostile environmental conditions. Breaking this concept, *Helicobacter pylori* was the first pathogen reported to inhabit the stomach. Recent studies have suggested that the stomach harbours transient as well as certain commensal bacterial and fungal species. The five major microbial phyla in the stomach have been identified as *Firmicutes*, *Bacteroidetes, Actinobacteria, Fusobacteria* and *Proteobacteria*.

The composition of gastric microbiota is dynamic and is affected by several factors. These include age group, dietary habits, medication use, inflammation of gastric mucosa and *H. pylori* colonization. Further, the role of host genetics has recently been studied in maintaining the stomach microbiota. Mutations in host genes may affect the host's immune response towards commensal bacteria and reduce their number and diversity.

The essential multiple roles of gut microorganisms include maintaining homeostasis in the gut, contributing to immune function and extraction of nutrients and energy from our diets. Loss of the normal balance between the gut microbiota and host has been associated with several abnormal conditions and disorders such as obesity, malnutrition, inflammatory bowel diseases (IBD), neurological disorders, and cancer. In the stomach, the interaction between *H. pylori* and the gastric microbiota can also influence gastric disease progression. Further studies should focus on addressing the role of gastric dysbiosis in health and disease. Identifying gastric microbiota is essential to understand how the gut microbiota and *H. pylori* affect health and disease.

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## Background

The gut microbiota is a complex ecological community, consisting of many microbes which include not only bacteria but also archaea, viruses, fungi and protozoa.[1,2] The gut microbiota consists of both commensal and pathogenic organisms residing in the gastrointestinal tract. Gut microbiota plays a central role in human health and has been studied extensively in recent times. The essential role of these microbiota includes nutrient and mineral absorption, immune function, synthesis of vitamins, enzymes and amino acids and also production of short chain fatty acids (SCFAs) which are being increasingly elucidated.[3,4] The byproducts, mainly acetate, propionate and butyrate of the fermentation processes by these organisms are important in maintaining the health of the human gut. For example, certain byproducts can be utilized to obtain energy by the gut epithelial cells.[5] Another important role of gut microbiota is its role in immunomodulation and providing protection against enteric pathogens. Gut microbiota therefore play an important role in maintaining the gut homeostasis and function.[6]

Dysbiosis is the alterations of composition and function of the gut microbiota. Various factors can lead to dysbiosis, including diet, antibiotic use, immunity, and an overly hygienic lifestyle.[7,8,9] In dysbiosis, the microbiota alters in its composition, showing reduced diversity and stability. In addition, there may also be increased levels of lipopolysaccharide-containing proinflammatory bacteria. Loss of normal balance between the gut microbiota and host has been associated with several diseases such as autoimmune disease, metabolic disorders (obesity and Type 2 diabetes), cardiovascular diseases (arthrosclerosis and hypertension), uremic diseases (chronic kidney disease), malnutrition, inflammatory bowel diseases (IBD), infectious diseases, neurological disorders, and cancer.[3,4]

Many studies have described the microbiota of the oral cavity and intestines while limited data is available on the microbial diversity of the stomach. Among the various microorganisms colonizing the gastrointestinal tract, *Helicobacter pylori* is recognized as a major pathogen responsible for chronic gastritis leading to peptic ulcer disease and gastric cancer.[10,11] The interaction between *H. pylori* and the microbiota also influences gastric disease progression. Therefore, identifying gastric microbiota is essential to understand how the gut microbiota and *H. pylori* affect health and disease. This review focus is on recent developments of the microbiota of the stomach and how they promote health and disease.

## Challenges in the colonization of the human stomach

The stomach is a formidable environment for microorganisms, mainly due to the acidic pH and the thickness of the mucus layer, and was long thought to be sterile. Furthermore, salivary nitrate enhances the antimicrobial properties of gastric juice by conversion to nitric oxide (NO) and reactive nitrogen intermediates in the stomach.[12] These NO radicals significantly kill ingested bacteria and are also involved in other gastroprotective mechanisms, such as regulation of mucosal blood flow and gastric mucus production.[13]

In 1983, the discovery of *H. pylori* in the gastric mucosa and its role in causing chronic gastritis established the stomach as an ecological niche for growth of microorganisms.[14] Later studies based on non-culture methods provided evidence for the presence of other genera of bacteria as well as yeasts in the human stomach.[15] Translocation of oral microbiota to the stomach and through the stomach to the lower part of gastrointestinal tract is reported and studies have suggested that translocation of oral bacteria to the intestines can cause dysbiosis and affect the immune defenses.[16,17]

Gastric juice contains mainly gastric acid and proteolytic enzymes (pepsins) which can inactivate microorganisms while initiating the process of digestion.[18] A study has shown that impaired gastric acid secretion results in higher susceptibility to infection and increased abundance of microbiota in the lower intestinal tract.[19] Thus, the gastric environment can have an impact on the microbial diversity of the gastrointestinal tract (GIT). The mucosa of the stomach acts as a physical barrier as well as a protective layer over the gastric epithelium.[20] The inner mucous layer is attached firmly to the epithelium while the outer mucous layer lies loosely over it. In addition, the gastric glands secrete antimicrobial mucins that can inhibit bacteria.[21,22] The stomach mucosal environment has also been shown to harbor antimicrobial peptides such as beta defensin 2, lysozyme, hepcidin and cathelicidin LL37.[23] The abundance of these compounds were found to vary in location within the stomach (gastric mucosa of fundus, corpus, and antrum). Colonizing the gastric mucosa therefore poses a considerable challenge due to the low pH in the gastric lumen, peristalsis action towards the ileum which hinders stable colonization of bacteria in the lumen, presence of the thick layer of mucous with antimicrobial compounds and reflux of bile and pancreatic secretions. Organisms that can survive this hostile environment can proceed further within the intestinal tract and find a more favorable environment in the intestines for colonization.[24]

**Diversity of gastric microbiota**

The composition of gastric microbiota is dynamic and is determined by factors such as dietary habits, medication use, inflammation of gastric mucosa and *H. pylori* colonization.[7] The composition of the gastric microbiota in different anatomical regions of the stomach is not understood completely. The human stomach contains only a few species of bacteria adhering to the mucous layer and most of them are transient flora.[25] The lack of bacteria in the stomach is attributed to the acidic environment and the presence of bile and pancreatic secretions, which kills most ingested microorganisms. Moreover, phasic propulsive motor activity towards the ileal end hinders the stable colonization of bacteria in the lumen.[26] Predominant genera of bacteria and fungi in different anatomical locations of the GI tract is shown in Figure 1.

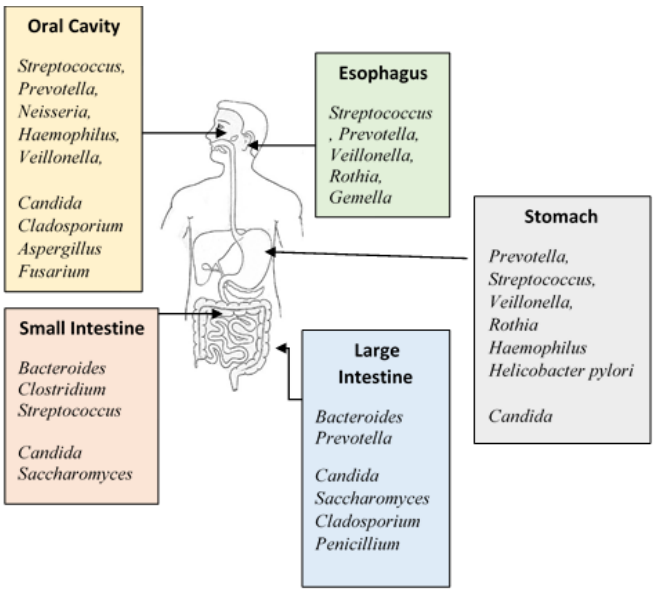


**Figure 1: Predominant genera of bacteria and fungi in different anatomical locations of the human GI tract [11, 26-30]**

Culture-based methods provide an incomplete and biased picture of the biodiversity of gastric microbiota, because more than 80% of microorganisms are uncultivable.[9,10] However, using culture-based methods, *Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria* and *Proteobacteria* have been detected as five major phyla in the stomach.[11] More than 65% of phylotypes identified by culture independent molecular methods in the stomach have been described in the human oral cavity. *Bacteroidetes* and *Firmicutes* usually dominate in adults.

*Actinobacteria* and *Proteobacteria* are frequently found in the stomach but generally in low density.[27]

Bik et al., (2006) analyzed gastric microflora from gastric samples using advanced molecular techniques (genomic sequence analysis using the 16S rDNA clone library approach) and identified five main phyla as *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria* and *Fusobacteria*. This confirms the findings of culture based identification.[26] The most abundant phyla in gastric mucosa were identified as *Firmicutes* and *Proteobacteria*.[28] Li et al. (2009) demonstrated the gastric microbiota of 10 healthy individuals by cloning and sequencing 16S rDNA, and identified 1233 non-*H. pylori* clones, 133 phylotypes and five dominant genera (*Streptococcus, Prevotella, Neisseria, Haemophilus, and Porphyromonas*).[29] Similarly, using culture and pyrosequencing techniques, gastric juice samples and gastric biopsies from twelve healthy individuals were analyzed. The most abundant genera found in this study were *Streptococcus, Propionibacterium, and Lactobacillus.*[30] Engstrand et al. (2013)[31] investigated the gastric microbiota of 13 healthy subjects by pyrosequencing and identified 200 phylotypes and five dominant genera *(Prevotella, Streptococcus, Veillonella, Rothia, Pasturellaceae).* These investigators did not find a significant difference in the microbial composition of the stomach antrum versus the corpus. In a study conducted in the pediatric population, four major phyla (*proteobacteria, bacteroidetes, firmicutes and actinobacteria*) were identified in the stomach by sequencing the V4 region of the 16S ribosomal RNA gene using high-throughput sequencing.[32] Culture-independent sequencing studies have demonstrated a vast microbial diversity that is highly variable, both over time and across human populations.

Gastric juice has been frequently used as the specimen in many studies addressing the microbial diversity of the stomach. It is possible that organisms such as *Veillonella*, *Lactobacillus* and *Clostridium* may be found transiently.[27] Gastric biopsy specimens have also been used to study the bacterial diversity. Delgado et al (2013)[30] reported *Streptococcus*, *Propionibacterium* and *Lactobacillus* as the most abundant species identified in gastric biopsy and gastric juice specimens and a similar microbial composition was observed across several different populations.

In Sri Lanka, *H. pylori* was identified using rapid urease (CLO) test in several studies.[33,34] In 2002, Fernando et al investigated the presence of *H. pylori* by PCR in gastric mucosal biopsies with a high prevalence of 70.1%.[35] Recent studies conducted in Sri Lanka have reported a lower proportion of *H. pylori* among dyspeptic patients.[35,36,37] However, there is limited data available on the gastric microbial diversity and related complications such as dysplasia, gastric cancer and the effects on both health and disease in Sri Lanka.

*Candida* species are part of oral microbiota and more attention has focused recently on the role of *Candida spp*. in dyspeptic patients. The stomach is a preferential niche for *Candida* colonization due to its acidic pH. However, it is observed that *Lactobacillus spp.* in gastric microbiota could promote resistance against pathogens such as *Candida spp.* by preventing adhesion of yeast cells and inhibiting hyphal invasion in the stomach.[38] Thus, dysbiosis in the gastric microbiome could lead to *Candida spp.* colonization in the stomach.

In 2001, Zwolińka-Wcisło et al. detected fungal colonization in 54.2% of patients with gastric ulcer and 10.3% of patients with chronic gastritis. In this study *Candida albicans* was found to be the most frequently isolated organism. *C. tropicalis* was also detected in the patients and control subjects in this study, while other species such as *C. glabrata, C. krusei, C.*

*parapsilosis, C. zeylanoides*, and *C. membranofaciens* were detected infrequently.[38] Rzewski et al. (2009), identified *C. albicans* in 30-50% of patients with chronic gastritis and ulcerous disease. *C. albicans* were isolated from gastric mucosal specimens by multi-locus sequence typing (MLST) suggesting a relationship between the susceptibility to dyspepsia and *C. albicans* genotypes.[39]

Low pH (pH=2) in the stomach is a primary factor for *C. albicans* being the dominant species in the gastric mucosa, though most other candida species cannot survive at this pH.[40] Of the 159 dyspeptic patients, 10 were found to harbor yeasts species in gastric biopsies by 26S rDNA sequencing [personnel communication and unpublished data]. Among these 10 patients, only 3 were positive for *H. pylori* although all ten patients had dyspeptic symptoms. This leads us to rethink and expand our knowledge on yeasts and its role in gastric pathology. However, *Candida spp.* colonization in gastric mucosa and its clinical relevance is not fully elucidated and is an important future research area.

**Role of gastric microbiota in health**

The gut microbiota promotes the fermentation of nondigestible substrates like dietary fibers and endogenous intestinal mucus. Fermentation supports the growth of microbes that produce short chain fatty acids (SCFAs) and gases. Acetate, propionate, and butyrate are main SCFAs produced during fermentation. Butyrate is a main energy source for human colonocytes, which activates intestinal gluconeogenesis and has beneficial effects on glucose and energy homeostasis.[41] It also induces apoptosis of colon cancer cells and is essential for epithelial cells to consume large amounts of oxygen through β oxidation, generating a state of hypoxia that maintains oxygen balance in the gut, preventing gut microbiota dysbiosis.[39] Propionate is transferred to the liver and regulates gluconeogenesis and satiety signaling through interaction with the gut fatty acid receptors.[42,43] Acetate is the most abundant SCFA and an essential metabolite for the growth of other bacteria. Acetate can reach the peripheral tissues where it is used in cholesterol metabolism and lipogenesis and may play a role in central appetite regulation.

Other specific products of the gut microbiota include trimethylamine and indole propionic acid. Trimethylamine is formed from dietary phosphatidylcholine and carnitine (from meat and dairy) and is dependent on gut microbiota and thus its amount in blood varies between people.[44] Trimethylamine is oxidized in the liver to trimethylamine N-oxide, which is positively associated with an increased risk of atherosclerosis and major adverse cardiovascular events. Indole propionic acid is highly correlated with dietary fibre intake which seems to reduce the risk of incidence of type 2 diabetes.

**Dysbiosis (microbial imbalance) and associated diseases**

In healthy individuals, there is cross-talk and cross-regulation between the host and the gut microbiota. This creates a homeostatic balance of bacteria preventing overgrowth of potentially pathogenic bacteria. The imbalance in the microbial equilibrium is termed "dysbiosis".[2] Dysbiosis can result from loss of beneficial organisms, or excessive growth of potentially harmful organisms, or loss of overall microbial diversity, or all these factors at the same time. These can result from exposure to various environmental factors, diet, toxins, drugs including antibiotics and pathogens.[44,45]

Studies done on experimental animal models suggest that enteric pathogens have the greatest potential to cause microbial dysbiosis. *H. pylori* is the most common bacterial pathogen worldwide which colonizes greater than 50% of the global population.[32] Several studies using

PCR- and sequencing-based approaches have demonstrated that *H. pylori*-negative individuals harbor a highly diverse gastric microbiota dominated by the five predominant phyla, *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes* and *Fusobacteria.*[45] *H. pylori* has been the single most abundant bacteria present in the stomach of *H. pylori* positive individuals in 72% and 97% of studies.[46] Studies had been done to compare the composition of gut microbiota with the progression of gastrointestinal disorders. Dicksved et al (2009)[21] showed that *H. pylori* was present in relatively low numbers in patients with advanced premalignant lesions and that the microbiota of patients with gastric cancer were dominated by species of *Lactobacillus, Streptococcus, Veillonella, and Prevotella.* Several studies suggest that the *Helicobacteraceae* family was significantly lower in the gastric cancer group, compared to patients with chronic gastritis and intestinal metaplasia.[21] Non-*H. pylori* bacteria contribute to pathogenesis of non-ulcer dyspepsia. Treatment of *H. pylori* infection with proton pump inhibitors can reduce gastric acid secretion and facilitate colonization by other bacteria.[47] Theisen et al. (2000) observed that PPI treatment for 14 days resulted in a significant increase in the total number of gastric bacteria.[48]

A recently published study by Guo et al (2020) showed the significant contribution of *H. pylori* to gastric dysbiosis which was restored following successful eradication therapy. Their study also suggests that certain *H. pylori* associated bacterial genera *(Fusobacterium*, *Neisseria*, *Prevotella*, *Veillonella* and *Rothia)* increased the risk of progression to precancerous lesions.[44]

The oral pathogen *P. gingivalis* is an anaerobic bacterium and is one of the main causes of periodontitis. It is thus easily accessible to the stomach as well. A study by Salazar et al (2013) reported that higher colonization of periodontal pathogens seen among patients with periodontal disease showed an increased risk of development of gastric precancerous lesions.[49] Another study demonstrated that *P. gingivalis* was not detectable in biopsy specimens from patients with gastric cancer whereas it was frequently found to be present in specimens from patients with esophageal cancer. This study also reported that the number of viable bacteria declined in the presence of acidic pH.[50] In another study, *Streptococcus* species were shown to be positively correlated with peptic ulcer disease. Patients with antral gastritis and atrophic gastritis have a higher number of *Streptococcus* species compared to healthy subjects.[51]

Diet is another key factor that influences the composition of the gut microbiota. A limited number of animal model studies have been conducted to understand the diversity of diet and effect of duration on the alterations in microbial composition.[52]

Certain environmental toxins can induce structural differences and functional alterations in the gut microbiome. It has been shown that exposure to xenobiotics such as antibiotics, heavy metals and artificial sweeteners induces gut microbiome toxicity.[53,54,55] Although many studies report on how antibiotic treatment alters gut microbiota, the effect on the gastric microflora is not widely reported.

Dysbiosis contributes to aberrant proinflammatory immune responses, susceptibility to invading pathogens and initiation of disease processes. In addition, dysbiosis is associated with intestinal disorders such as inflammatory bowel disease, irritable bowel syndrome (IBS)[56] and coeliac disease and extra-intestinal disorders like allergy, asthma, metabolic syndrome, cardiovascular disease and obesity.[3,4,57] Low microbial diversity is considered as a

marker of dysbiosis in the GIT. A lower diversity of microbes has also been observed in autoimmune diseases, obesity and cardiometabolic conditions, as well as in elderly people.[58]

**The role of host genes involved in microbiota regulation**

The role of host genetics has been recently appreciated in maintaining the GIT microbiota. The GIT microbiome is initially shaped by maternal transmission during birth, then further influenced by various other factors. A direct role for GIT microflora in the maturation and function of the immune system has been reported in humans and it has been shown that part of the microbiome is heritable, suggesting that host genetics are an important factor in determining gut microbiome composition.[58] In humans, several host genetic variants were tested for their association with microbiome composition and function. Genetic variants in FUT2 genes (fructosyltransferase 2) were associated with microbial energy metabolism and mucosal inflammation. MEFV gene polymorphisms were associated with major shifts in bacterial phyla. The various mutations in the MEFV gene amplify the host's immune response towards commensal bacteria and reduce their number and diversity.[59] Therefore, even polymorphisms in genes encoding proteins that are not supposed to be in direct contact with the GIT microbes can influence host–microbe interactions.[49] A Sri Lankan study reported the heterozygous gene mutations in MEFV gene among the dyspeptic population. In this study, three common heterozygous mutations (E148Q, P369S, M680I) were investigated and it was concluded that the MEFV gene was not an important determinant contributing to the presence of *H. pylori* in a dyspeptic population.[33]

## Conclusion

The gastric microbiota is not fully understood compared to the intestinal and oral microbiota. The composition of microbes contributing to a 'healthy' gut microbiota is still an enigma. Many recent studies suggest that the healthy human stomach holds a microbiome dominated by *Prevotella, Streptococcus, Veillonella, Rothia* and *Haemophilus*. However, the role of these and other microbes, except for *H. pylori* on health and disease yet remains to be elucidated. *Candida albicans* has been identified in patients with gastric pathology, suggesting a possible role as a stomach pathogen. Further studies are needed to understand the role of *Candida albicans* as a gastric pathogen.

Future studies using molecular based techniques to determine the composition of gastric microbiota, and its role in health and disease are needed to better understand the gaps in knowledge. The maintenance of microbial homeostasis is essential for the stomach's health. Factors that play an essential role in maintaining stomach microbial homeostasis such as diet, environmental agents, antibiotics and host genes should be targeted in future research.

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