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Review Article

Is Stomach a Sterile Environment?

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Abstract

The stomach is considered a sterile organ for a long time due to anatomical and physiological features, till the discovery of *Helicobacter pylori* in 1982, which demolished the conception of sterile stomach. The pathogenicity of *Helicobacter pylori* is enhanced by several virulence factors. Initially, with the aid of culture-based techniques which were later followed by advanced culture-independent molecular techniques, whereby the complexity and biodiversity of gastric microbiota were revealed. Commensals, as well as pathogenic microbes have developed mechanisms to ensure successful colonization in the gastric environment. A number of published literature suggests the correlation of these bacteria with gastric diseases including gastric cancer and peptic ulcer disease as well as the beneficial relationships like probiotics. This review summarizes current information on the correlation of complexity and diversity of gastric microbiota and host in health and disease.

Keywords: Stomach, *Helicobacter pylori,* Gastric cancer, Gastric microbiota

Introduction

The stomach is a unique environment with many anatomical and physiological adaptations for its functions of mechanical digestion and initiation of chemical digestion of protein components of food. This organ was considered as a sterile organ for a long period of time. This long prevailed scientific dogma was demolished by the discovery of *Helicobacter pylori* (*H. pylori*) in 1982. For several years, it was considered that only this bacterium could survive in the gastric environment. However, the recent advancement of culture-based techniques as well as culture-independent molecular techniques made provisions for the available knowledge on the complexity and the diversity of the gastric microbiome.

The complexity and diversity of microbiota are indicated by the presence of 10-100 trillion microbial cells throughout the human body.[1] The knowledge of gastric microbiota is indispensable in understanding the pathogenesis, diagnosis, and treatment of gastric pathologies. This article reviews the current knowledge on human gastric microbiota, highlighting the correlation between structural and functional adaptations of microbiota and gastric diseases.

Anatomical and physiological factors that help to maintain sterility in the stomach

The hostile surrounding of the stomach created by low pH, gastric rugae, peristalsis, mucus thickness and bile acid reflux, was thought to be inhibiting the colonization of microbes.

Gastric acid is a major factor that contributes to the gastric bactericidal barrier which plays a vital role in the digestion of food in the stomach by creating a low pH environment within the range of pH 1.5 to 3.5. In 1972, an in-vitro study was conducted on the bactericidal activity of normal gastric juice, which revealed that at pH less than 4.0, 99.9 % of the bacteria get killed within a period of 30 minutes.[2]

Gastric rugae are coiled tissue sections in mucosa and submucosa in the stomach that contribute to the antibacterial environment of the stomach. A study conducted in 2010 suggests that gastric infections affect the integrity of gastric folds. In this study, 36 equine stomachs were examined for lesions caused by bacterial infections. Hyperplastic rugae were observed in 13 stomachs in this study and intracellularly Escherichia-like bacteria had been observed. This bacterial species was *Escherichia fergusonii* which is an emerging pathogen in both humans and animals.[3] Gastric peristalsis contributes to the sterility of the stomach by the mechanical removal of microbes with the aid of muscle movements. An experiment conducted on the elimination of *Vibrio cholerae (V. cholerae)* from the gastrointestinal tract of adult mice revealed the effect of peristalsis on the elimination of *V. cholerae* by varying the factors affecting peristalsis. This study depicted that the rapid removal of microorganisms by peristalsis has a significant effect on the reduction of a viable number of microorganisms.[4]

Bile acids are biological detergents that facilitate emulsification and solubilization of dietary lipids and also display potent antimicrobial activity as the bacterial membranes become their main targets. De Valdez, using electron microscopy revealed that strains of *Lactobacillus reuteri* exposed to bile showed severe distortion of the cell envelope and membrane alterations that presented folds and buds.[5]

Helicobacter pylori

The unexpected discovery of *H. pylori* was a remarkable finding in the history of microbiology. In 1982, Warren and Marshall observed flagellated, spiral or curved bacilli in biopsy specimens from antral mucosa. This Gram-negative, flagellated, and microaerophilic bacterium was initially named as *Campylobacter pyloridis* by Warren and Marshall due to its close resemblance to the genus *Campylobacter* in respect of atmospheric requirements and DNA base composition. However, their

flagellar morphology is not that of the genus *Campylobacter*.[6] Hence in 1984, the bacterium was renamed as *Helicobacter pylori* by the two scientists. For this revolutionizing discovery that changed the perspective of gastric pathologies, Warren and Marshall received the Nobel Prize in Physiology or Medicine in 2005.

Survival Mechanism of *Helicobacter pylori*

H. pylori possesses unique characteristics that aid in the 3 major pathogenic processes, including colonization, immune escape, and disease induction. Motility, urease production and adhesion factors help the bacteria to penetrate, colonize and survive in unfavorable highly acidic gastric environment. The motility of *H. pylori* is conferred by two to six sheathed unipolar flagella that extend 3-5 μm from the bacterial surface, with bulb-like structures often seen at the tip of the filaments. In 1996, a study was conducted to determine if one or both flagellin genes, FlaA and FlaB are necessary for colonization or persistence by *H. pylori* in gnotobiotic piglets. It was observed that non-motile mutants lacking flagella were unable to establish persistent infection in the animal model used.[7]

Urease enzyme helps the bacterium to resist gastric elimination effectively by neutralizing the extreme acidity. The role of urease in the pathogenesis of gastritis induced by *H. pylori* was experimented using gnotobiotic piglets, which revealed that *H. pylori* survives at a pH range between 4.0 and 8.0 in the absence of urea. However, in the presence of urea the organism can survive at a pH as low as 2.5.[8]

Several outer membrane proteins (Hop proteins) of *H. pylori* have been identified as adhesion factors, which facilitate the adherence of the bacterium to the gastric epithelium. These include proteins like BabA, SabA, OipA, AlpA, AlpB etc. The mechanism of adhesion factors in pathogenicity of *H. pylori* infection is still not fully understood. A study conducted in 2011 revealed that *H. pylori* strains expressing low levels of BabA contributed to more severe mucosal injury and were more frequently associated with duodenal ulcer and gastric cancer than strains with a high-level expression of BabA or those lacking the BabA gene.[9]

Once established, *H. pylori* expresses virulent proteins such as Cytotoxin-associated gene A (CagA) and Vacuolating cytotoxin A (VacA) that control the host's immune system to escape immune detection and allow its persistence in the human stomach.

Non-*H. pylori* Organisms

Over 3 decades it was believed that only *H. pylori* was able to survive the hostile gastric environment. But further investigations revealed the complexity of microbial community residing in association with the stomach and the duodenum. These investigations discovered that in addition to *H. pylori*, there are non-pylori Helicobacter bacterial strains as well as non-Helicobacter strains in the stomach. Prior to the discovery of *H. pylori*, a report published in 'The Lancet' in 1981 revealed the presence of several acid-resistant bacterial strains in the stomach. These included *Streptococcus, Neisseria,* and *Lactobacillus*.[10] Since the discovery of *H. pylori*, over 20 species of *Helicobacter* have been officially recognized through many studies.

In 2006, Bik et al. used a small subunit 16S rDNA clone library approach to study the bacterial diversity within the human gastric mucosa, by analyzing gastric biopsy samples of 23 individuals. It was observed that *H. pylori*, the only member of the genus Helicobacter constituted 42% of all sequences analyzed. Subsequent to *H. pylori*, *Streptococcus* sp. (299 clones) and *Prevotella* sp. (139 clones) were observed as the most abundant species.[11]

A study conducted in 2013 used culturing and pyrosequencing of gastric juice and biopsy specimens of 12 healthy individuals revealed that Streptococcus, Propionibacterium and Lactobacillus were the most abundant genera

among gastric microbiota.[12]

In 2014, Khosravi et. al studied culturable bacteria in the stomach among a large population of 215 Malaysian patients referred for endoscopy and revealed that Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes are the major phyla in human gastric microbiota.[13]

There is limited data available on diversity of gastric microbiota among Sri Lankan population. Previous studies conducted on the prevalence of *H. pylori* among Sri Lankan dyspeptic patients showed a low prevalence due to low sensitivity of methods used.[14] However, in 2002, Fernando et al. using PCR, observed that there was 75.4% prevalence of *H. pylori* among 57 Sinhalese dyspeptic pateints.[14]

A recent study conducted in Sri Lanka, has identified yeast species and bacteria (including *H. pylori*) in the gastric mucosa of patients with dyspepsia. The same study reported that out of 70 gastric biopsy specimens, yeast was found in 10 specimens while bacterial species were found in 65 specimens. Also, the study reported 14 cases of *H. pylori* infection by doing a rapid identification test (IBUT) and histological examination.[15]

In the same study, bacterial DNA when subjected to Denaturing Gradient Gel Electrophoresis (DGGE), reported multiple bands in a single specimen suggesting the presence of multiple species in the gastric mucosa in patients with dyspepsia. Further interestingly, through DGGE and band sequencing techniques it was found that most of the yeast species found in gastric mucosa were *Candida albicans*. [personnel communication and unpublished data]

Techniques Associated with Discovery of Gastric Microbiota

Culture Based Techniques

In early years, the identification process of bacteria was solely dependent on culture-based techniques. These methods have paved the way to many important investigations such as identification of bacterial diversity of the stomach. Mucosal biopsy and gastric juice specimens were mostly used for culturing. Studying gastric juice alone can lead the investigator to underestimate the diversity of bacterial microbiota in the stomach as it does not calculate the inhabitants of mucosal membrane. Furthermore, the identification of bacterial strains by conventional culture-based methods provides an incomplete and biased picture of the biodiversity of gastric microbiota, as more than 80% of microorganisms are uncultivable.[16] However, it has been argued that culture-based techniques provide an advantage over molecular methods in distinguishing viable microorganisms.[16]

Culture Independent Molecular Techniques

To overcome disadvantages of culture-based technologies, culture-independent molecular methods based on 16S rRNA gene became effective[12]. Among the molecular based studies dot-blot hybridization with rRNA targeted probes, targeted qPCR fluorescent in situ hybridization (FISH), traditional or sequence-aided community fingerprinting including denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) ,terminal restriction fragment length polymorphism (T-RFLP), sequencing of cloned 16S rDNA, microarrays (PhyloChip) and next-generation sequencing (NGS) have been used to determine the diversity of gastric microbiota.[12]

Disease Associated with Gastric Microbiota

Chronic Gastritis

H. pylori infections produce various degrees of chronic inflammation in gastric mucosa. Several studies suggest that *H. pylori* colonization leads to formation of mucosa-associated lymphoid tissue lymphomas (MALTomas) and aggregation of polymorphonuclear leucocytes. This leads to histological changes compatible with gastritis. In 2009, Li et al. revealed that the over-

representation of the *Streptococcus* genus within the Firmicutes phylum, can lead to histological change of gastritis of the stomach, even in the absence of *H. pylori*.[17] This indicates the role of other microbiota in gastroduodenal diseases.

Peptic Ulcer Disease

Peptic ulcer disease can be considered as a complication of chronic *H. pylori* infection. Genetic variability and diverse virulence factors such as CagA and VacA can determine various levels of risk for duodenal or gastric peptic ulcers.[18] Loss of parietal cells effects in decreased gastric acid output creates a favorable environment, allowing the other microbial communities to colonize. In 2014, Khosravi et al. demonstrated a significant correlation between the isolation of *Streptococci* and peptic ulcer disease.[13] Accordingly, non-*H. pylori* bacteria may also play a vital role in the pathogenesis of gastroduodenal diseases.

Gastric Cancer

H. pylori is considered to be a fundamental cause of most gastric malignancies. In a prospective cohort study conducted in 2007 using 1,225 dyspeptic Taiwanese, among which 618 were *H. pylori* infected, it was concluded that *H. pylori* infected patients are more susceptible to develop gastric malignancies, including adenocarcinoma and lymphoma.[19] Furthermore, World Health Organization (WHO) has classified *H. pylori* as a carcinogen. In 1994, the International Agency for Research on Cancer (IARC), a subordinate organization of the WHO, identified *H. pylori* as a 'group 1 (definite carcinogen)' based on the results of epidemiologic studies.[20]

Other Infections

It has been proved by many studies that acute gastrointestinal infections can activate irritable bowel syndrome.[21] The correlation between the gastroduodenal microbiota and colonic neoplasia has been studied worldwide. In 2012, Zhang et al. observed that among patients with colorectal cancer *H. pylori* infection was more prevalent than among controls.[22]

Mechanism of Colonization of Gastric Microbiota

Nutrients

The colonization of species is diverse according to their metabolism in the gut. The colonization ability of gut bacteria is determined by the ability to utilize a specific nutrient which can be a limiting factor. In 2013, a study conducted using gnotobiotic mice identified commensal colonization factors (CCFs) which are bacterial species-specific carbohydrate utilization systems, in *Bacteroides vulgatus* and *Bacteroides fragilis*. The bacteria are capable of colonizing in suitable nutrient niches with the aid of CCFs.[23]

Mucus and Adherence

In the gut, both commensals and pathogens, to colonize should reach epithelium by overcoming the mucosal barrier and the immune system. The bacterial motility is restricted by flagellin which is immunogenic as it is a ligand for Toll-like Receptor 5 (TLR5). The other limiting factor of bacterial motility is the viscosity of mucus. A study conducted in 2010 revealed that *Shigella flexneri* and *E. coli* have developed a strategy of secreting Pic, a mucin-binding serine protease that rapidly digests mucus. Furthermore, this protein interferes with the ability of indigenous bacteria to compete with the pathogen by stimulating hypersecretion of mucus.[24]

Antimicrobials

Paneth cells, a specialized immune cell type lying at the base of the crypts of the small intestine with the ability of secreting cationic antimicrobial peptides which restrict the growth of bacteria in the mucosal surface. Several Gram-negative pathogens have developed modifications in lipid A, a major component of the outer membrane in order to develop resistance against antimicrobials. A study showed that a modification in lipid A of

H. pylori by under phosphorylation, was found to be important for resilient colonization by *Bacteriodes thetaiotaomicron* in inflammation.[25]

Factors Affecting Diversity of Gastric Microbiota

Socio-demographic factors including age, gender, ethnicity, and dietary behaviors can influence the diversity of gastric microbiota.[26] It has being observed that the microbiota undergoes considerable changes in infants and older people.[27] In the elderly population, it was observed that changes in the composition of Firmicutes and increases in the proportion of Bacteroidetes.[27]

The correlation of gastric microbiota with gender was revealed in several studies. In 2006, a cross-sectional study on the composition of gut microbiota among 230 European subjects suggested that gender affects the distribution of microbiota, with the observation of males having higher levels of the *Bacteroides-Prevotella* group than females.[28]

When considering the effect of ethnicity on the diversity of gastric microbiota, a comparative study conducted by evaluating the fecal microbiota between different geographic locations or different ethnic groups has found large variation in specific bacterial groups.[29]

Dietary patterns and diet composition are believed to strongly influence the diversity of gut microbiota. The correlation of gastric microbiota composition with diet and health was studied among 178 elderly subjects. It was observed that the abundance of short-chain fatty acid producing bacteria is affected by the quality and diversity of the diet.[27]

Plant materials used as herbs and spices in processing food can affect the diversity of gut microbes. A study conducted on bactericidal and antiadhesive properties of 25 culinary and medicinal plants against *H.pylori,* demonstrated that turmeric was observed the most efficient in killing *H.pylori* which is followed by cumin, ginger, chili, borage, black caraway, oregano and liquorice. Furthermore, it was observed that extracts of turmeric, borage, and parsley were able to inhibit the adhesion of *H pylori* strains to the stomach sections.[30]

Health Benefits of Gut Microbiota

Despite the presence of pathogenic gut microbiota, there can be beneficial commensals that help in the physiological processes of the host and prevent colonization of pathogenic organisms like *Candida spp*. that has been detected in stomach. However, it has been observed that *Lactobacillus sp*. can inhibit such pathogens.[26] Hence Lactobacilli can be used as probiotic to manufacture dairy products preventing *H.pylori* infection.

The roles of the gut microbiota in resisting the colonization of enteric pathogens, promoting the maturation of the host immune system, and host metabolism, as depicted by many studies in the past years, provide an explanation to mechanisms underlying the vast supportive roles of the gut microbiota in human health. The ability of gut commensals to inhibit pathogen colonization is mediated via several mechanisms including direct killing, competition for limited nutrients, and enhancement of immune responses. A study conducted in 2014 reported the occurrence of innate immune defects in germ-free mice resulting from the absence of gut microbiota. It was observed that recolonization of germ-free mice with a complex microbiota restores the immune defects and develops resistance to systemic infection with *Listeria monocytogenes*.[13]

Summary

The discovery of *H. pylori* demolished the scientific dogma that 'the stomach is a sterile organ'. Several studies suggest that the healthy human stomach holds a core microbiome including *Prevotella*, *Streptococcus*, *Veillonella*, and *Haemophilus*. The adaptations to neutralize acidity, overcome mucus barrier, and ability to

escape the host's immunity enable the survival and colonization of gastric microbiota. It was evident that microbial interactions influence an individual's risk of gastric diseases, including gastric cancer.

Further, studies on the composition of gastric microbiome and their role in health and disease are required to address the variations of bacterial diversity in the extremely acidic environment. Changes in gastric acidity and the use of probiotic or antibiotic therapies need to be attentively analyzed for their effect on the structure and function of the gastric microbiome. There could be many other mechanisms of the gut microbiota in causing the host systemic infections that are yet to be discovered.

Conclusion

Gastric microbiota plays a vital role in the development of gastric disorders. Further studies on complexity and diversity of gastric microbes will benefit in clear understanding of their correlation with health and disease. The available knowledge on this topic is confined to a few species. In addition to individual pathogenic species, microbes can act synergistically to develop diseases.

Conflict of Interest

The authors have no conflict of interest to declare

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