GASTRIC MICROBIOTA: TRACING THE CULPRIT

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Abstract

The gastric environment has been long time considered bacteria-free, but the discovery of Helicobacter pylori (H. pylori) in 1982 superseded this conception. Over the last decades new diagnostic methods have been developed, starting with culture-dependent and advancing to culture-independent ones. These modern techniques provide new insight into the composition and influence of this ecosystem on the entire gastrointestinal tract. H. pylori is no longer considered the only microorganism in the stomach, other non-H. pylori microbial species may populate the same environment and exercise their role. Current knowledge suggests possible links of these bacteria with gastroduodenal diseases, such as peptic ulcer and gastric cancer but most of them need further scientific evidence. This review summarizes current information on these complex interrelations between gastric microbial communities and host in health and disease.

Keywords: gastric microbiota, microbiome, stomach, Helicobacter pylori

Introduction

The stomach, with its low pH, had been considered for many years a sterile organ. The discovery of Helicobacter pylori (H. pylori) in 1982 was revolutionary in many aspects and challenged the previous concepts in digestive pathology. After the isolation and identification of H. pylori, advances in both culture-dependent and culture-independent techniques have been made. Serological assays, temperature gradient gel electrophoresis, next-generation sequencing or metabolomic and proteomic studies are just some of the approaches which have tried to describe as extensively as possible the structure and function of these microbial communities. Indeed, this complex and dynamic biodiversity of bacteria comprises as much as 10-100 trillion microbial cells in different parts of the body [1]. Regarding the gastrointestinal tract, modern techniques have documented a bacterial load of $10^{10}$ to $10^{12}$ colony-forming units (CFU)/mL in the colon, considerably higher than that of the stomach, where it reaches $10^{2}$ to $10^{4}$ CFU/mL [2].

In 2000 Lederberg predicted that the human microbiota would become a hot research topic worldwide and time has indeed proven him right [3]. Although a large pool of data has been gathered until now, understanding the complex interaction between our bodies and our microbial communities is still in the beginning. The lower gastrointestinal tract microbiota has long been the subject of intensive studies, and lately the upper gastrointestinal environment research has been catching up, as reflected in published literature. The stomach is a unique environment which influences the rest of the microbiome through its local acidic conditions and resident microbial communities.

The pathogenesis, diagnosis and treatment of gastric illnesses will be better understood if we “read” well the resident biodiversity of bacteria. This article highlights the current knowledge of the human gastric microbiota by describing its structure, dynamics and interactions with gastrointestinal and extra-digestive diseases.

The Gastric Environment

The stomach presents particular anatomical features that cause a particular composition of bacteria, different from that of bacteria in other GI segments. Acid secretion, the reflux of bile, mucus thickness and gastric peristalsis all contribute to the formation of this strong antimicrobial
environment. However, the discovery of *H. pylori* in 1982 and further investigations with modern techniques put an end to the traditional view of the sterile stomach.

There is a dynamic equilibrium of the gastric bacterial density in relation to the local pH fluctuations. The human gastric juice pH varies from 1-2 interprandial to >5 after food ingestion [4]. Therefore, the time and site of sampling are of great importance, as the species structure tends to be different in the stomach corpus than in the antrum [5]. A comprehensive systematic review on gastric acidity across 68 species, has revealed that humans seem to have a gastric juice pH closer to carrion feeders than to most carnivores and omnivores and that the stomach acts as an ecological filter for microbial communities prior to entering the intestines [6].

Exposure to other environmental factors, such as dietary habits and medication use, also plays a very important role in the composition of the gastric milieu. Despite numerous reports of dietary effects on the structure of the intestinal microbiota, little is known about the impact of diet on the gastric biodiversity. An animal model study compared the colonization of *Lactobacilli* in the stomachs of mice fed purified and non-purified diets [7] and found higher populations of microbiota in the latter, among which *Lactobacilli* were predominant. Nakae et al. treated 44 patients with functional dyspepsia (FD) and 44 healthy controls with a yogurt containing a probiotic strain of *Lactobacillus gasseri* OLL2716 (LG21 yogurt) and investigated the effects on the gastric bacteriological parameters and symptoms [8]. The study found significant dysbiosis in the microbiota of patients with FD compared to controls and that dysbiosis was restored after treatment with LG21 yogurt with subsequent decrease in the gastric fluid volume and amelioration of symptoms. New treatment regimens using a more personalized approach for gastrointestinal disorders are expected to be developed in the near future.

The use of antacid therapy has increased significantly over the last decade and this leads to important changes in gastric microbial biodiversity [9]. Acid suppressive therapy causes increase in gastric pH, which allows more microbial communities to colonize the gastric environment. From this viewpoint, a study in 2015 using 16S rRNA gene profiling documented changes in the microbiota of the gastric fluid in proton pump inhibitors (PPI)-users and PPI-nonusers and suggested a reduced bacterial clearance in PPI-users [10]. Likewise, Paroni et al. [11] using 16S rRNA gene pyrosequencing in patients with dyspepsia, showed that subjects treated with omeprazole exhibited different gastric microbial communities from untreated patients. Interestingly, Ahn and colleagues conducted a meta-analysis which reported that acid suppressive drugs are associated with an increased risk of gastric cancer [12]. Further prospective studies are needed in order to quantify more accurately the real impact of antacid medication on gastric diseases.

The administration of antibiotics fundamentally alters normal gastrointestinal microflora. A culture-independent and culture-dependent study of the murine gastric microbiota showed that cefoperazone treatment perturbed the microbial communities of the stomach, causing overgrowth of *Enterococci* and a reduction in the number of *Lactobacilli* [13]. Rosenvinge et al. described the bacterial and fungal microbiota in the stomach fluid from 25 patients using PCR amplification of bacterial 16S rRNA genes and fungal internal transcribed spacers and concluded that antibiotics reduced bacterial, but not fungal biodiversity [14]. Future research is expected to lead to more specific drugs against pathogens, ensuring protection of the healthy microbiome and minimal damage to symbiotic bacteria.

**Helicobacter pylori**

The discovery of *Helicobacter pylori* changed the long-held traditional view of the stomach as a sterile organ and improved our understanding of how microbial communities survive under hostile acidic environment. *H. pylori* is a Gram-negative, spiral shaped, motile and flagellated Epsiliprotoebacteria that belongs to the family Helicobacteraceae and colonizes the gastric mucosa of at least 50% of the worldwide population. The prevalence of *H. pylori* shows large geographical variations and ranges between 25-37% in Western Europe, Australia, Oceania and the Northern America, with a rapidly decreasing trend, and up to 70-87% in Africa, Western Asia and South America, being correlated with multiple geographical and infrastructural factors [15]. In developing countries, infection is usually acquired early in childhood, unlike in industrialized countries, where it develops more commonly in adulthood [16].

To colonize the gastric epithelial layer and produce disease, *H. pylori* possesses peculiar characteristics which make it a special gastric pathogen [17]. The helical morphology, motile ability, adhesion factors, urease and ammonia production help the bacteria penetrate, colonize and survive in such an unfavorable acidic environment. Once established, it generates a complex inflammatory response that injures the gastric mucosa and determines the subsequent digestive diseases. The virulence of *H. pylori* is expressed through various markers of pathogenicity, such as cytotoxin-associated gene A (CagA), BabA adhesin and vacuolating cytotoxin (VacA), which represent the new focus of the current research into the development of gastric diseases [18].

*H. pylori* has the highest relative abundance among all gastric microbial communities when present in adults [19,20]. Another study that aimed to describe the gastric microbiota in pediatric patients once again confirmed that when present, *H. pylori* tends to prevail over the rest of the microbial ecosystem [21]. Importantly, the sensitivity across the methods of detection varies, 16S rRNA studies
identified *H. pylori* DNA in patients who were negative upon PCR testing [22].

An important feature of *H. pylori* is its great genetic diversity, which stems from a high mutation rate and a constant exchange of genetic material with their human host [23]. Various strains have been isolated from different locations worldwide, suggesting that *H. pylori* has coevolved with humans throughout history. The heterogeneous interaction between the bacterium, the host, and the environment influences the clinical outcome and may lead to either disease or possible protective effects, especially regarding esophageal adenocarcinoma [24]. Advances in metagenomics might determine researchers to reconsider current knowledge and elaborate a more personalized therapeutic approach.

**Non-Hp Gastric Microbiota**

The widely held view that *H. pylori* species were the only organisms capable to survive in the hostile gastric environment has been assumed over the last 3 decades. Further investigations overturned this concept and revealed a far more complex landscape in which different microbial communities reside in the stomach and the duodenum. Indeed, the gastric microbial density is now estimated at around $10^2$ to $10^4$ colony-forming units (CFU)/mL, with variations related to local pH, food ingestion, medication and site of isolation [2]. Due to a median pH of 1.4 in the gastric lumen, it is, however, considerably lower than compared to the microbial density of the colon, where it reaches $10^6$ to $10^{12}$ CFU/mL.

In order to identify the microorganisms in the stomach several different methodologies were developed. Initially, conventional methods such as gastric juice cultures and mucosal biopsies documented the presence of various gastric microorganisms and considered them as transient bacteria, which form small colonies that exist for short periods of time rather than true gastric colonizers [25,26]. Another culture-based study reported *Clostridium* spp, *Veillonella* spp and *Lactobacillus* spp as the most predominant gastric species in the normal acidic stomach [27]. However, these techniques underestimate the biodiversity of bacteria, as a great part of them cannot be cultured [28].

As a consequence several culture-independent molecular techniques have been developed. A pioneer study using temperature gradient gel electrophoresis of PCR-amplified 16S rDNA fragments showed that *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Pseudomonas* and *Stomatococcus* are the most abundant genera [29]. Although several of those bacteria inhabit the oral cavity and respiratory tract, the documentation of *Pseudomonas* spp other than *Paeruginosa* led to the idea that the gastric milieu exhibits an indigenous microbiota. By using a small subunit 16S rDNA clone library approach, Bik et al. analysed gastric biopsy samples and identified 128 phylotypes, which fell into five different bacterial phyla: *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria* [19]. The wide bacterial diversity in this gastric environment was significantly different from that observed in the oral cavity and the esophagus, indicating that the human stomach may be home to a distinct microbial community. In 2013, Delgado et al. conducted the first study in which a combination of classical culturing and culture-independent techniques was used and found that the most abundant genera belonged to *Streptococcus*, *Lactobacillus* and *Propionibacterium* [2].

Recent interesting work tried to identify a possible influence of host genetic backgrounds on gastric microbiota by analyzing antral biopsies from four pairs of twins and eight unrelated individuals, but concluded that co-twins did not have higher similarity in the biodiversity of gastric bacteria [30].

The development of modern techniques [31], such as whole-genome sequencing, fluorescence in situ hybridization, metabolomic and transcriptomic analyses of the bacteria, may offer a deeper understanding of the gastric ecosystem and its role in health and disease. These studies, applied initially to the gut environment [32], should move beyond identifying bacteria towards a functional characterization and understanding of the complex interrelationship between microorganisms and hosts.

**Relationship between Hp and Other Gastric Microbiota**

Microbial communities in the human body interact both with their host and also with each other. The relation between *H. pylori* and other gastric microbiota seems to be far more complex than originally thought. Research on the bacterial biodiversity within the stomach in *H. pylori* positive versus *H. pylori* negative gastric mucosa is still controversial.

Several studies revealed that *H. pylori* is capable to modify its own microclimate. This may be explained by various factors. *H. pylori* is known to produce ammonia and bicarbonate from urea which may serve as substrates for other microbial communities. Additionally, it reduces the gastric secretion and thus elevates the pH in the stomach, generating favorable ecological niches for the colonization of other microorganisms. *H. pylori* also induces the production of cytokines and antimicrobial peptides that cause chronic gastric inflammation and may inhibit other local microorganisms [33].

From this viewpoint Osaki et al. [34] documented an abundance of *Eubacterium cylindroides* and *Prevotella* species and a decrease of *Bifidobacterium*, *Clostridium cocoides* and *Clostridium leptum* species in *H. pylori*-negative, but not in *H. pylori*-positive Mongolian gerbils. Another study performed on 12 subjects once again confirmed that human gastric microbial ecosystem showed important differences according to *H. pylori* status [22]. Interestingly, other reports also show that certain species such as *Lactobacillus* exhibit high antagonistic effects.
and could inhibit the growth of *H. pylori* [35]. Similarly, *Streptococcus mitis*, a commensal bacteria of the gastric environment, is likely to induce growth inhibition and coccoid conversion from a spiral form of *H. pylori* cells [36].

However, other published data on the murine gastric microbiota [37] suggests that neither acute nor chronic *H. pylori* infection substantially modifies the gastric microbial ecosystem. Khosravi et al. [38] found no significant differences in the composition of the gastric microbiota between 131 *H. pylori*-positive and 84 *H. pylori*-negative individuals.

Several factors may account for the heterogeneity of these results, such as the time of *H. pylori* infection, the degree of mucosal inflammation and the different methods used to diagnose the infection of *H. pylori* and other bacteria. As the structure of the gastric ecosystem depends on a plethora of factors, further experiments are required to determine the exact relationship between *H. pylori* and other gastric microbiota and to achieve better knowledge of its function in health and disease.

**Gastric microbiota in Relation to Gastroduodenal Diseases**

**Chronic Gastritis and Peptic Ulcer Disease**

*H. pylori* infection produces various degrees of chronic inflammation of the underlying mucosa with only a subgroup to develop clinical manifestations and further pathological changes in the stomach [39]. The etiologic link between longstanding *H. pylori* and chronic gastritis is well-documented [40,41]. Despite fewer studies addressing the role of other microbiota in gastroduodenal diseases, it seems that different gastric microbial communities, such as the over-representation of the *Streptococcus* genus within the *Firmicutes* phylum, can lead to gastritis as well, even in the absence of *H. pylori* [42].

Peptic ulcer disease is a recognized complication of chronic *H. pylori* infection, with 95% of duodenal and 70% of gastric ulcers being linked to it [43]. *H. pylori*'s genetic variability and diverse virulence factors (such as cagPAI) can determine various levels of risk for duodenal or gastric peptic ulcer [17]. Low acid output is a consequence of the loss of parietal cells and allows other microbial communities to colonize the gastric environment. A further study demonstrated a significant correlation between the isolation of *Streptococci* and peptic ulcer disease [38]. Accordingly, non-*H. pylori* bacteria may also play an important role in the pathogenesis of gastroduodenal diseases, through complex mechanisms and interactions that remain to be fully clarified.

**Gastric cancer**

Gastric cancer is the fifth most common malignancy in the world and the third leading cause of cancer death [44]. *H. pylori* was the first bacterium to be considered carcinogenic and represents the main etiologic agent in non-cardia gastric cancer, causing approximately 90% of such cases globally [45]. This infection promotes gastric carcinogenesis through the Correa cascade of inflammation, gastric atrophy, intestinal metaplasia and dysplasia in a subset of cases. A significant correlation between the presence of *H. pylori* and the development of gastric cancer was found in several prospective studies [46,47,48], all leading to the idea that *H. pylori* is a necessary cause of most gastric malignancies. At the same time, the eradication of *H. pylori* reduces the risk of gastric cancer development, according to several international consensuses [49,50].

However, *H. pylori* coevolved with humans for millennia and only 1% to 2% of persons infected with these bacteria actually develop severe complications, such as gastric cancer or MALT-lymphoma [51]. Other bacterial, host and environmental factors were also associated with the increased susceptibility to gastric cancer [52]. From this viewpoint, specific *H. pylori* strains [53], host genetic susceptibility [54], hyperglycemia [55], smoking [56], diet [57] and other microbiota may also contribute to the outcome of infection.

An increasing pool of evidence suggests that other microbial communities play a causative role in the pathophysiology of gastric cancer. These non-*H. pylori* bacteria that overgrow in a hypoacidic environment could potentiate carcinogenesis through various mechanisms, such as promoting inflammation, stimulating cell proliferation, modifying stem cell dynamics and producing toxic metabolites [58]. Animal studies reported an inhibition in the development of gastrointestinal intraepithelial neoplasia in germ-free INS-GAS mice compared to *H. pylori*-infected INS-GAS mice containing a complex gastric microbiota [59]. To date, there are few studies investigating the effects of non-*H. pylori* communities on gastric carcinogenesis. Eun and colleagues found a greater diversity of gastric microbiota in the gastric cancer group in comparison to other chronic gastritis and intestinal metaplasia groups, especially in *H. pylori* – positive patients [60]. A recent study by Yu et al. [61] using 16 S ribosomal RNA gene sequencing analysis and PICRUSt bioinformatics software package suggested a possible role of the gastric biodiversity of bacteria in gastric cardia carcinogenesis. Another study performed on two human populations with high and low gastric cancer risk in Columbia reported two significantly more abundant operational taxonomic units (OTUs), *Leptotrichia wadei* and *Veillonella sp.*, in the high-risk area and 16 OTUs, including *Staphylococcus sp.*, more frequent in the low-incidence region [62]. In addition, no significant correlation of the gastric biodiversity with *H. pylori* phylogeographic population or carriage of the cagPAI was documented in this study.

However, in other human studies [63], no significant role of bacteria other than *H. pylori* was found in the gastric carcinogenesis. A further study revealed that the diversity of
gastric microbiota actually decreased along the progression from non-atrophic gastritis to intestinal metaplasia and intestinal-type gastric cancer [64] and ranged from 57 genus in the first group to 8 genus in the last one. Given the emerging importance of novel biocomputational tools in the assessment of the structure and interactions of gastric microbiota, future studies on this issue are required.

Other implications

Interplay between gastric microbiota and different gastrointestinal and extra-digestive diseases is of great interest in many recent studies. Epidemiologic reports have provided strong evidence that there is a causal link between *H. pylori* infection and gastric MALT lymphoma [65], and that the eradication of this bacteria confers excellent long-term outcome of the disease [66]. Acute gastrointestinal infection seems to be the trigger for post-infectious irritable bowel syndrome [67] and post-infectious functional dyspepsia [68], according to several studies. The correlation between *Helicobacter pylori* and colorectal neoplasia was underscored by many large-scale studies over the last years [69,70] which confirmed that this bacterial infection confers an increased risk for colonic neoplasm.

Regarding the extra-gastrointestinal involvement, reports found possible associations between gastric microbiota (especially *H. pylori*) and hematological diseases like idiopathic thrombocytopenic purpura [71] and anemia [72], cardiovascular [73], neurological [74], endocrine [75] and even dermatological diseases [76]. Moreover, eradication of *H. pylori* seems to have beneficial effects on many aspects of these diseases [77,78].

There has been a conceptual shift over the last decades from the theory of a sterile stomach towards the view of a far more complex and dynamic gastric ecosystem, with resident microbial communities that permanently interact with each other and the host. When present, *H. pylori* represents the main colonizer but it is certainly not the only one. In order to better characterize the composition of this harsh environment, several technical methodologies and even specific devices [79] have been developed, pushing the medical limits further and further.

This review focused on the potential harmful impact of this complex ecological system but the whole picture seems to be far more nuanced. Increasing evidence emerges showing possible protective effects of *H. pylori* and other gastric bacteria. The loss of our indigenous microbial flora could lead to the increase in modern allergic and metabolic diseases, as reflected in the “disappearing microbial flora” hypothesis [80,81]. From this viewpoint, some studies found that the presence of *H. pylori* in the gastric milieu exhibits an inverse relationship with esophageal adenocarcinoma [82], asthma [83] and obesity [84]. Current research directions investigate the possible benefits of infecting people with benign strains of *H. pylori* [85].

In order to reconcile both of these aspects, we should cautiously interfere with the structure of the microbiome, trying to eradicate only bacteria that lead to inflammation and disease. To sum up, even if substantial progress in identifying the culprit has been made, the judgement cannot be absolute, as we should circumscribe it only to its unfavorable effects and allowing it to exercise its role as the body attempts to maintain homeostasis.

Conclusions

The stomach may accommodate bacteria, the best known of them being *Helicobacter pylori*, involving different and potentially harmful pathological conditions. Other bacteria may coexist in the stomach. Practitioners should be aware of the bacterial colonization of the stomach and the indications to eradicate these infections.

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