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The human gastric microbiota: Is it time to rethink the pathogenesis of stomach diseases?

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Abstract

Introduction: Although long thought to be a sterile organ, due to its acid production, the human stomach holds a core microbiome.

Aim: To provide an update of findings related to gastric microbiota and its link with gastric diseases.

Methods: We conducted a systematic review of the literature.

Results: The development of culture-independent methods facilitated the identification of many bacteria. Five major phyla have been detected in the stomach: *Firmicutes*, *Bacteroidites*, *Actinobacteria*, *Fusobacteria* and *Proteobacteria*. At the genera level, the healthy human stomach is dominated by *Prevotella*, *Streptococcus, Veillonella*, *Rothia* and *Haemophilus*; however, the composition of the gastric microbiota is dynamic and affected by such factors as diet, drugs and diseases. The interaction between the pre-existing gastric microbiota and *Helicobacter pylori* infection might influence an individual's risk of gastric disease, including gastric cancer.

Conclusions: The maintenance of bacterial homeostasis could be essential for the stomach's health and highlights the chance for therapeutic interventions targeting the gastric microbiota, even if gastric pH, peristalsis and the mucus layer may prevent bacteria colonization; and the definition of gastric microbiota of the healthy stomach is still an ongoing challenging task.

Keywords

Gastric cancer, gastric microbiota, gastritis, *Helicobacter pylori*, review, stomach bacteria, stomach cancer

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Introduction

Interest in the microbiologic composition of the normal digestive tract started in the 19th century, when Louis Pasteur stated that "life without bacteria would be impossible." A few years later, Elie Metchnikoff postulated that lactic acid bacteria have health benefits capable of promoting longevity. Today, humans are considered superorganisms constituted by cells and symbiotic microorganisms, although the number of microorganisms that colonize the human body is 10 times the number of human body cells, and the number of microorganism genes is 150 times the number of human genes.[1] Therefore, research on human diseases must include the microbiome, to obtain a complete picture of a given condition.

In 2000, the editors of *Science* prophesized that "human microbe research will become the new hot topic worldwide."[2] In 2007, the US National Institute of Health (NIH) launched the Human Microbiome Project.[3] Just 1 year later, the European Union (EU) funded a project devoted to the metagenomics of the human intestinal tract. Finally, in 2009, the International Human Microbiome Consortium began to explore the relationship between microbiota and human health and disease.[4,5] Since then, a huge amount of data, prevalently on gut microbiota, has been produced; and the time seems ripe to draw together the various research threads. Here we review the latest findings related to the composition of the gastric microbiota, the factors that modulate it, and the

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relationship between gastric microbiota and *Helicobacter pylori* (*H. pylori*) infection.

Composition of the gastric microbiota

Research on gastric microbiota laid dormant for many years, mainly as a consequence of the dogma that, due to its acid production, 'the stomach is a sterile organ' inhospitable to bacteria. In addition, the reflux of bile acids in the stomach, the thickness of the mucus layer and the effectiveness of gastric peristalsis might have impeded bacterial colonization of the stomach. Furthermore, the nitrate contained in saliva and food is converted by *Lactobacilli* present in the mouth into nitrite that, once in the stomach, is transformed by gastric juice into nitric oxide, a strong antimicrobial agent. All these factors, together with technical difficulties in collecting samples for analysis, and the lack of simple, reliable diagnostic tests have hampered the challenging study of the gastric microbiota.[6,7]

The discovery of *Campylobacter pyloridis* by Robin Warren and Barry Marshall in 1982 demolished the dogma of the sterile stomach. The bacterium, renamed *H. pylori* in 1984, colonizes and damages the gastric mucosa by a complexity of bacterial mechanisms. *H. pylori* escapes gastric elimination, due to the urease enzyme, which produces ammonia from urea. Ammonia neutralizes acid, thereby enabling the bacterium to penetrate the mucus layer, colonize the epithelium and foster the complex inflammatory response that damages the gastric mucosa and leads to chronic gastritis in the majority of infected people, or to peptic ulcer in a subgroup (10%), and to gastric malignancies in a minority (<1%).

In 1981, a few months before the discovery of *H. pylori*, *The Lancet* reported that a large number of acid-resistant bacterial strains are detectable in the stomach, among which are *Streptococcus, Neisseria* and *Lactobacillus*. The presence of these bacteria is not surprising, as the stomach is exposed to swelling of bacteria from the oral cavity and reflux of bacteria from the duodenum. More than 65% of phylotypes identified in the stomach have been described in specimens from the human mouth.[8] Thus, such species of bacteria as *Veillonella*, *Lactobacillus* and *Clostridium*, that are found in gastric juice may just be transient.[9]

Transient bacteria establish small colonies for brief periods of time, without colonizing the gastric mucosa, and do not cross talk with the host; however, whether bacteria other than *H. pylori* colonize the gastric mucosa and cross-talk with the host by penetrating the thick mucus layer, is not known. Thus, the study of the gastric juice alone for the presence of bacteria is not conclusive, and it may underestimate the real presence of bacteria at the mucosal level. Indeed, while *Firmicutes, Bacteroidetes and Actinobacteria* dominate the gastric fluid samples, *Firmicutes* and *Proteobacteria* are the most abundant phyla in gastric mucosal samples.[10] In addition, the identification of bacterial strains by conventional culture-based methods provides an incomplete and biased picture of the biodiversity of gastric microbiota, because more than 80% of microorganisms are uncultivable.[11,12] Recently, the development of culture-independent molecular methods based on 16S rRNA genes, such as fluorescent in situ hybridization, dot-blot hybridization with rRNA-targeted probes, denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, and cloning and sequencing of rDNA have facilitated the identification and classification of gastrointestinal bacteria.[13]

Bik et al.[10] analyzed for the first time the gastric mucosa of 23 healthy adult subjects using a small subunit 16S rDNA clone library approach: They identified 1056 non-*H. pylori* clones, 127 phylotypes and five dominant genera (*Streptococcus, Prevotella, Rothia, Fusobacterium* and *Veillonella)*. A few years later, Li et al.[14] analyzed the gastric microbiota of 10 healthy subjects, by cloning and sequencing 16S rRNA, and identified 1223 non-*H. pylori* clones, 133 phylotypes and five dominant genera (*Streptococcus, Prevotella, Neisseriae, Haemophilus* and *Porphyromonas*). In 2013, Engstrand et al.[15] investigated the gastric microbiota of 13 healthy subjects by pyrosequencing, identifying 200 phylotypes and five dominant genera (*Prevotella*, *Streptococcus, Veillonella, Rothia, Pasturellaceae*), and they did not differ by comparing antrum versus body.

In the same year, Delgado et al.[16] analyzed gastric juice and gastric biopsy samples of 12 healthy subjects by culturing and pyrosequencing, and found that the most abundant genera were *Streptococcus*, *Propionibacterium* and *Lactobacillus*. Although these studies examined different populations (African-American, Hispanic, Chinese and European subjects), the gastric microbiota at both the phyla and genera level, was surprisingly similar in all of them, even if with a large degree of inter-subject variability.

Factors affecting the composition of gastric microbiota

The composition of gastric microbiota at the genera level is dynamic and is affected by such factors as dietary habits, medication use, inflammation of gastric mucosa and, of course, *H. pylori* colonization. While many studies document the effect of diet on gut microbiota composition in humans,[17–21] there are only a few with evidence, mainly limited to animal model studies, addressing the effects of diet on gastric microbiota. An in vivo study shows higher levels of total aerobes, total

anaerobes, and *Lactobacilli* in the stomach of mice fed a non-purified diet (natural source-derived food), with respect to mice fed a purified diet (refined food). This increase correlates with lower levels of Toll-like receptor 2 (TLR-2) mRNA in the stomach.[22]

The long-term use of proton pump inhibitors (PPIs) and H_2-antagonists, as well as atrophic gastritis, affects the composition of the gastric microbiota; this is not surprising, considering that gastric microbiota depends on gastric acid secretion. Bacterial overgrowth occurs when the gastric pH was >3.8.[23] Oro-pharyngeal-like bacteria and fecal-like bacteria are significantly more abundant in patients on PPI therapy than in patients on H_2-antagonists and untreated control subjects.[24] Treatment for 2 weeks with PPI reduces gastric acid secretion by 75% and this was sufficient to permit bacterial colonization of the stomach in healthy volunteers.[23] Omeprazole (40 mg/day) for 3 months induced gastric bacterial overgrowth in 10 of 30 patients, compared with 1 of 10 control subjects; however, after only 14 days of PPI treatment (omeprazole 30 mg/day), the total number of gastric bacteria had increased to a significant level.[25]

Antibiotics are known to have negative ecological effects on gastrointestinal microflora. Animal studies show that penicillin treatment reduces *Lactobacilli* populations and promotes yeast colonization of the gastric epithelium. Using culture-dependent and culture-independent approaches, Mason et al.[26] demonstrated that cefoperazone treatment in the human causes long-term alteration of gastric microbiota, such as a significant reduction in the number of *Lactobacilli* and overgrowth of *Enterococci*.

Relationship between Helicobacter pylori *and gastric microbiota*

Studies in animal models show that long-term *H. pylori* infection affects the bacterial composition of the gastric microbiota. Indeed, an abundance of *Eubacterium cylindroides* and *Prevotella* species and a decrease of *Bifidobacterium* species, *Clostridium coccoides* and *Clostridium leptum* were found in *H. pylori*-negative, but not in *H. pylori*-positive gerbils.[27] Studies conducted in patients without *H. pylori* infection reveal that they have a relative lack of *Proteobacteria* and *Bacteroidetes* phyla, and a relative abundance of *Streptococcus* and *Prevotella* genera.[14] In *H. pylori*-positive patients, with respect to *H. pylori*-negative subjects, Maldonado-Contreras et al.[28] report a higher abundance of *Proteobacteria*, *Spirochetes* and *Acidobacteria*; and a decreased abundance of *Actinobacteria*, *Bacteroidetes* and *Firmicutes*.

H. pylori-induced changes in gastric microflora can be attributed to various factors. Long-term *H. pylori* infection leads to gastric atrophy, and consequently, to an increase of the gastric pH, which prompts the colonization of the stomach by transient bacteria. In addition, ammonia and bicarbonate produced by *H. pylori* from urea may serve as substrates for other bacteria. Finally, *H. pylori* slows gastric motility, thereby favoring the clearance of adherent bacteria from the mucosa.[9] It is likely that *H. pylori* creates special niches that allow the survival and colonization of bacteria in the stomach. Notwithstanding these findings, the relationship between *H. pylori* and the gastric microbiota is still controversial. Indeed, Tan et al.[29] report that chronic *H. pylori* infection does not significantly alter the microbiota of the murine stomach. Similarly, Bik et al.[10] did not find significant differences in gastric microbiota between *H. pylori*-positive and *H. pylori*-negative patients, in terms of 16S rDNA sequences. Very recently, Khosravi et al.[30] confirmed these findings in a large sample of subjects, including 131 *H. pylori*-positive and 84 *H. pylori*-negative patients.

It is likely that factors such as the degree of *H. pylori*-related inflammation; the time of the infection; and the presence, type and extension of precancerous lesions have to be taken into account, when dealing with the composition of gastric microbiota.

Gastric microbiota and the diseased stomach

Animal and human studies indicate that gastric colonization by bacteria that normally colonize the lower bowel could affect the outcome of *H. pylori* infection and the risk of gastric cancer.[31–34]

In C57BL/6 N mice grown in different environments, i.e. Charles River Lab and Taconic Farms, and then infected with the same strain of *H. pylori*, the rate of inflammation, gastritis and metaplasia was significantly greater in those mice grown in Charles River Lab. Interestingly, the rate of gastric colonization by *Lactobacillus* strains differed significantly between the two groups of mice.[35] These data are consistent with the idea that mice from different vendors will have different resident stomach microbial populations.

In addition, germ-free transgenic insulin-gastrin (INS-GAS) mice, a mouse model of gastric cancer, developed gastric intraepithelial neoplasia with a marked time delay when infected with *H. pylori* alone, compared with those infected with *H. pylori* plus a normal complex gastric microbiota.[36] Similarly, the colonization of the stomach by an artificial intestinal microbiota ('Altered Schaedler's Flora', including *Clostridium* species, *Lactobacillus murinus* and *Bacteroides* species) increased the incidence of gastric intraepithelial neoplasia up to 69% in male INS-GAS mice, 7 months after *H. pylori* infection.[37]

Finally, antibiotic treatment significantly delayed the onset of gastric neoplasia in *Helicobacter*-free and specific pathogen-free INS-GAS mice.[38]

A culture-based study of 103 biopsies from *H. pylori*-infected patients showed that the colonization with non-*H. pylori* bacteria was higher in the non-ulcer dyspepsia group than in the gastric ulcer group ($p < 0.001$), which suggests a pathogenetic role of non-*H. pylori* bacteria in non-ulcer dyspepsia.[39] In addition, a positive correlation was observed between *Streptococcus* and peptic ulcer disease.[30]

Patients with antral gastritis, compared with *H. pylori*-negative subjects, show a decrease of *Proteobacteria* and an increase of *Firmicutes* at the phyla level, and a significant increase of *Streptococcus* at the genera level, without significant differences between the gastric antrum and body, in both groups.[14] Similarly, patients with atrophic gastritis, with respect to healthy subjects, had an increase of *Streptococcus* and a decrease of *Prevotella*.[15]

Recently, a gradual shift in the gastric microbiota profile from non-atrophic gastritis to intestinal metaplasia, and then to intestinal-type gastric cancer, was reported.[40] Lower microbial richness (number of bacterial genera per sample) in the upper digestive tract was independently associated with a lower serum pepsinogen I/pepsinogen II ratio, which corresponds to a cancer-predisposing state in the stomach.[41] Culture-based analysis of patients with gastric cancer reveals more microorganisms and more anaerobic bacteria (e.g. *Clostridium* and *Bacteroides* species) than those from normal mucosa.[42] Patients with cancer had a relative abundance of *Streptococcus*, *Lactobacillus*, *Veillonella* and *Prevotella*; and a decrease of the *H. pylori* strain.[42] Interestingly, *Veillonella* favors the accumulation of nitrite in the stomach, thus promoting a carcinogenic effect.[39] In this context, it is noteworthy that the concentration of nitrite is significantly higher in the gastric juice of patients with gastric cancer, than in controls.[43]

Very recently, a 454 GS FLX Titanium-based analysis of the microbial composition, diversity and richness in gastric mucosa from patients with chronic gastritis, intestinal metaplasia and gastric cancer found a relative increase in the bacilli class in the gastric cancer group, compared with the other groups. Moreover, the *Helicobacteraceae* family was significantly lower in the gastric cancer group, compared with chronic gastritis and intestinal metaplasia groups, while the relative abundance of *Streptococcaceae* family significantly increased. In addition, the denseness and diversity of gastric microbiota in the gastric cancer group increased, compared with other groups.[44]

Overall, these data suggest that, in animal models, and also probably in humans, the gastric microbiota can affect the immunobiology of the gastric mucosa, and as a consequence, the outcome of *H. pylori* infection and the related carcinogenic process.[45] Nevertheless, a unique bacterial profile has not yet been identified, and the mechanistic link to cancer needs to be clarified.

Conclusions

The discovery of *H. pylori* demolished the historical dogma of medicine that 'the stomach is a sterile organ'. Current data suggest that the healthy human stomach holds a core microbiome dominated by *Prevotella*, *Streptococcus, Veillonella*, *Rothia* and *Haemophilus*. A shift in abundance of *Firmicutes* phylum and the *Streptococcus* and *Prevotella* genera can be found in the *H. pylori*-infected stomach and in gastric cancer. The pharmacological inhibition of gastric acid secretion and gastric atrophy enable the survival and proliferation of other microbes that are normally killed by acid.

Our understanding of gastric microbiota has increased in recent years, but it is still unclear if and to what extent *H. pylori* infection perturbs the gastric microbiota, and if and to what extent the gastric microbiota modulates the outcome of an *H. pylori* infection. Nevertheless, it is becoming evident that their interaction influences an individual's risk of gastric disease, including gastric cancer. Further studies in humans that aim to clarify the composition of gastric microbiota, and its role in health and disease, will be welcome.

What are the next steps?

Future studies concerning gastric microbiota should include microbial and metabolomic profiles, correlated with the natural history of specific diseases. Subsequently, germ-free animals could be used to understand the casual relationship between bacteria and host disease. Once this step is reached, the possibility of modulating the gastric microbiota with the aim to change the natural course of diseases, or to prevent them, could be addressed.

Advances in molecular technologies would allow obtaining more data from a reduced amount of samples at a lower cost, which will further enable more detailed studies of the stomach microbiome, and will make data available to a broader range of scientists.

Unraveling the molecular basis of bacteria-host interactions will improve probiotic selection for different clinical indications.

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Conflict of interest

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