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environment has been long time considered bacteria-free, but the discovery of Helicobacter pylori (H. pylori) in 1982 superseded this conception.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 but also 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 Epsilonproteobacteria 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^{10} 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 *P.aeruginosa* 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 coccoides* 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 cytotoxin-associated gene A and duodenal ulcer promoting gene A) 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 microbiota" 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.

References

1. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev. 2012;70 Suppl 1:S38-S44.
2. Delgado S, Cabrera-Rubio R, Mira A, Suárez A, Mayo B. Microbiological survey of the human gastric ecosystem using culturing and pyrosequencing methods. Microb Ecol. 2013;65(3):763–772.
3. Lederberg J. Infectious history. Science. 2000;288:287–293.
4. Hunt RH, Yaghoobi M. The Esophageal and Gastric Microbiome in Health and Disease. Gastroenterol Clin North Am. 2017;46(1):121–141.
5. Gall A, Fero J, McCoy C, Claywell BC, Sanchez CA, Blount PL, et al. Bacterial Composition of the Human Upper Gastrointestinal Tract Microbiome Is Dynamic and Associated with Genomic Instability in a Barrett's Esophagus Cohort. PLoS One. 2015 Jun 15;10(6):e0129055.
6. Beasley DE, Koltz AM, Lambert JE, Fierer N, Dunn RR. The evolution of stomach acidity and its relevance to the human microbiome. PLoS One. 2015 Jun 15;10(7):e0134116. doi: 10.1371/journal.pone.0134116.
7. Sahasakul Y, Takemura N, Sonoyama K. Different impacts of purified and nonpurified diets on microbiota and toll-like receptors in the mouse stomach. Biosci Biotechnol Biochem. 2012;76(9):1728–1732.
8. Nakae H, Tsuda A, Matsuoka T, Mine T, Koga Y. Gastric microbiota in the functional dyspepsia patients treated with probiotic yogurt. BMJ Open Gastroenterol. 2016;3(1):e000109.
9. Fisher L, Fisher A. Acid-Suppressive Therapy and Risk of Infections: Pros and Cons. Clin Drug Investig. 2017;37(7):587-624.
10. Tsuda A, Suda W, Morita H, Takanashi K, Takagi A, Koga Y, et al. Influence of Proton-Pump Inhibitors on the Luminal Microbiota in the Gastrointestinal Tract. Clin Transl Gastroenterol. 2015 Jun 11;6(6):e89. doi: 10.1038/ctg.2015.20.
11. Paroni Sterbini F, Palladini A, Masucci L, Cannistraci CV, Pastorino R, Ianiro G, et al. Effects of Proton Pump Inhibitors on the Gastric Mucosa-Associated Microbiota in Dyspeptic Patients. Appl Environ Microbiol. 2016;82(22):6633–6644.
12. Ahn JS, Eom CS, Jeon CY, Park SM. Acid suppressive drugs and gastric cancer: a meta-analysis of observational studies. World J Gastroenterol. 2013;19(16):2560–2568.
13. Mason KL, Erb Downward JR, Falkowski NR, Young VB, Kao JY, Huffnagle GB. Interplay between the gastric bacterial microbiota and Candida albicans during postantibiotic

recolonization and gastritis. Infect Immun. 2012;80(1):150–158.

14. von Rosenvinge EC, Song Y, White JR, Maddox C, Blanchard T, Fricke WF. Immune status, antibiotic medication and pH are associated with changes in the stomach fluid microbiota. ISME J. 2013;7(7):1354–1366.

15. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;53(2):420-429. doi: 10.1053/j.gastro.2017.04.022.

16. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev. 2006;19(3):449–490.

17. Schulz C, Schütte K, Malfertheiner P. Helicobacter pylori and Other Gastric Microbiota in Gastroduodenal Pathologies. Dig Dis. 2016;34(3):210–216.

18. Backert S, Neddermann M, Maubach G, Naumann M. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2016;21 Suppl 1:19–25.

19. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103(3):732–737.

20. Schulz C, Schütte K, Koch N, Vilchez-Vargas R, Wos-Oxley ML, Oxley APA, et al. The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection. Gut. 2016 Dec 5. doi: 10.1136/gutjnl-2016-312904. [Epub ahead of print]

21. Llorca L, Pérez-Pérez G, Urruzuno P, Martinez MJ, Iizumi T, Gao Z, et al. Characterization of the Gastric Microbiota in a Pediatric Population According to Helicobacter pylori Status. Pediatr Infect Dis J. 2017;36(2):173–178.

22. Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, Karaoz U, Contreras M, Blaser MJ, et al. Structure of the human gastric bacterial community in relation to Helicobacter pylori status. ISME J. 2011;5(4):574–579.

23. Correa P, Piazuelo MB. Evolutionary History of the Helicobacter pylori Genome: Implications for Gastric Carcinogenesis. Gut Liver. 2012;6(1):21-28.

24. Dorer MS, Talarico S, Salama NR. Helicobacter pylori's unconventional role in health and disease. PLoS Pathog. 2009;5(10):e1000544. doi: 10.1371/journal.ppat.1000544.

25. Savage DC. Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol. 1977;31:107–133.

26. Stark CA, Adamsson I, Edlund C, Sjösted S, Seensalu R, Wikström B, et al. Effects of omeprazole and amoxycillin on the human oral and gastrointestinal microflora in patients with Helicobacter pylori infection. J Antimicrob Chemother. 1996;38(6):927–939.

27. Zilberstein B, Quintanilha AG, Santos MA, Pajecki D, Moura EG, Alves PR, et al. Digestive tract microbiota in healthy volunteers. Clinics (Sao Paulo). 2007;62(1):47–54.

28. Vartoukian SR, Palmer RM, Wade WG. Strategies for culture of 'unculturable' bacteria. FEMS Microbiol Lett. 2010;309(1):1–7.

29. Monstein HJ, Tiveljung A, Kraft CH, Borch K, Jonasson J. Profiling of bacterial flora in gastric biopsies from patients with Helicobacter pylori-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis. J Med Microbiol. 2000;49(9):817–822.

30. Dong Q, Xin Y, Wang L, Meng X, Yu X, Lu L, et al. Characterization of Gastric Microbiota in Twins. Curr Microbiol.

2017;74(2):224-229.

31. Propheter DC, Hooper LV. Bacteria Come into Focus: New Tools for Visualizing the Microbiota. Cell Host Microbe. 2015;18(4):392–394.

32. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, et al. Quantitative Imaging of Gut Microbiota Spatial Organization. Cell Host Microbe. 2015;18(4):478–488.

33. Mustapha P, Paris I, Garcia M, Tran CT, Cremniter J, Garnier M, et al. Chemokines and antimicrobial peptides have a cag-dependent early response to Helicobacter pylori infection in primary human gastric epithelial cells. Infect Immun. 2014;82(7):2881–2889.

34. Osaki T, Matsuki T, Asahara T, Zaman C, Hanawa T, Yonezawa H, et al. Comparative analysis of gastric bacterial microbiota in Mongolian gerbils after long-term infection with Helicobacter pylori. Microb Pathog. 2012;53(1):12–18.

35. Delgado S, Leite AM, Ruas-Madiedo P, Mayo B. Probiotic and technological properties of Lactobacillus spp. strains from the human stomach in the search for potential candidates against gastric microbial dysbiosis. Front Microbiol. 2015 Jan 14;5:766.

36. Khosravi Y, Dieye Y, Loke MF, Goh KL, Vadivelu J. Streptococcus mitis induces conversion of Helicobacter pylori to coccoid cells during co-culture in vitro. PLoS One. 2014 Nov 11;9(11):e112214. doi: 10.1371/journal.pone.0112214.

37. Tan MP, Kaparakis M, Galic M, Pedersen J, Pearse M, Wijburg OL, et al. Chronic Helicobacter pylori infection does dot significantly alter the microbiota of the murine stomach. Appl Environ Microbiol. 2007;73(3):1010–1013.

38. Khosravi Y, Dieye Y, Poh BH, Ng CG, Loke MF, Goh KL, et al. Culturable bacterial microbiota of the stomach of Helicobacter pylori positive and negative gastric disease patients. ScientificWorldJournal. 2014;2014:610421. doi: 10.1155/2014/610421.

39. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. J Clin Invest. 2004;113(3):321–333.

40. de Vries AC, Kuipers EJ. Helicobacter pylori infection and nonmalignant diseases. Helicobacter. 2010;15 Suppl 1:29–33.

41. Engstrand L, Lindberg M. Helicobacter pylori and the gastric microbiota. Best Pract Res Clin Gastroenterol. 2013;27(1):39–45.

42. Li XX, Wong GL, To KF, Wong VW, Lai LH, Chow DK, et al. Bacterial microbiota profiling in gastritis without Helicobacter pylori infection or non-steroidal anti-inflammatory drug use. PLoS One. 2009 Nov 24;4(11):e7985. doi: 10.1371/journal. pone.0007985.

43. Ford AC, Gurusamy KS, Delaney B, Forman D, Moayyedi P. Eradication therapy for peptic ulcer disease in Helicobacter pylori-positive people. Cochrane Database Syst Rev. 2016 Apr 19;4:CD003840. doi: 10.1002/14651858.CD003840.pub5.

44. Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM FD, Bray F. International Agency for Research on Cancer. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide. IARC CancerBase. 2012; No. 11. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on day/month/year

45. Plummer M, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer. 2015;136(2):487–490.

46. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, et al. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BMJ. 1991;302(6788):1302–1305.

47. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med. 1991;325(16):1132–1136.

48. Hsu PI, Lai KH, Hsu PN, Lo GH, Yu HC, Chen WC, et al. Helicobacter pylori infection and the risk of gastric malignancy. Am J Gastroenterol. 2007;102(4):725–730.

49. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut. 2012;61(5):646–664.

50. Sheu BS, Wu MS, Chiu CT, Lo JC, Wu DC, Liou JM, et al. Consensus on the clinical management, screening-to-treat, and surveillance of Helicobacter pylori infection to improve gastric cancer control on a nationwide scale. Helicobacter. 2017 Jun;22(3). doi: 10.1111/hel.12368.

51. Herrera V, Parsonnet J. Helicobacter pylori and gastric adenocarcinoma. Clin Microbiol Infect. 2009;15(11):971–976.

52. Brawner KM, Morrow CD, Smith PD. Gastric microbiome and gastric cancer. Cancer J. 2014;20(3):211–216.

53. Enroth H, Kraaz W, Engstrand L, Nyrén O, Rohan T. Helicobacter pylori strain types and risk of gastric cancer: a case-control study. Cancer Epidemiol Biomarkers Prev. 2000;9(9):981–985.

54. Persson C, Canedo P, Machado JC, El-Omar EM, Forman D. Polymorphisms in inflammatory response genes and their association with gastric cancer: A HuGE systematic review and meta-analyses. Am J Epidemiol. 2011;173(3):259–270.

55. Sheu SM, Cheng H, Kao CY, Yang YJ, Wu JJ, Sheu BS. Higher glucose level can enhance the H. pylori adhesion and virulence related with type IV secretion system in AGS cells. J Biomed Sci. 2014;21:96.

56. González CA, López-Carrillo L. Helicobacter pylori, nutrition and smoking interactions: their impact in gastric carcinogenesis. Scand J Gastroenterol. 2010;45(1):6–14.

57. Raei N, Behrouz B, Zahri S, Latifi-Navid S. Helicobacter pylori Infection and Dietary Factors Act Synergistically to Promote Gastric Cancer. Asian Pac J Cancer Prev. 2016;17(3):917–921.

58. Abreu MT, Peek RM Jr. Gastrointestinal malignancy and the microbiome. Gastroenterology. 2014;146(6):1534–1546.e3. doi: 10.1053/j.gastro.2014.01.001.

59. Lofgren JL, Whary MT, Ge Z, Muthupalani S, Taylor NS, Mobley M, et al. Lack of commensal flora in Helicobacter pylori–infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. Gastroenterology. 2011;140(1):210–220.

60. Eun CS, Kim BK, Han DS, Kim SY, Kim KM, Choi BY, et al. Differences in gastric mucosal microbiota profiling in patients with chronic gastritis, intestinal metaplasia, and gastric cancer using pyrosequencing methods. Helicobacter. 2014;19(6):407–416.

61. Yu G, Hu N, Wang L, Wang C, Han XY, Humphry M, et al. Gastric microbiota features associated with cancer risk factors and clinical outcomes: A pilot study in gastric cardia cancer patients from Shanxi, China. Int J Cancer. 2017;141(1):45-51.

62. Yang I, Woltemate S, Piazuelo MB, Bravo LE, Yepez MC, Romero-Gallo J et al. Different gastric microbiota compositions in two human populations with high and low gastric cancer risk in Colombia. Sci Rep. 2016 Jan 5;6:18594. doi: 10.1038/srep18594.

63. Jo HJ, Kim J, Kim N, Park JH, Nam RH, Seok YJ, et al. Analysis of Gastric Microbiota by Pyrosequencing: Minor Role of Bacteria Other Than Helicobacter pylori in the Gastric Carcinogenesis. Helicobacter. 2016;21(5):364–374.

64. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, Mantilla A, Torres J. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. Sci Rep. 2014;4(1):4202. doi: 10.1038/srep04202.

65. Du MQ, Atherton JC. Molecular subtyping of gastric MALT lymphomas: implications for prognosis and management. Gut. 2006;55(6):886–893.

66. Nakamura S, Sugiyama T, Matsumoto T, Iijima K, Ono S, Tajika M, et al. Long-term clinical outcome of gastric MALT lymphoma after eradication of Helicobacter pylori: a multicentre cohort follow-up study of 420 patients in Japan. Gut. 2012;61(4):507–513.

67. Ishihara S, Tada Y, Fukuba N, Oka A, Kusunoki R, Mishima Y, et al. Pathogenesis of irritable bowel syndrome--review regarding associated infection and immune activation. Digestion. 2013;87(3):204–211.

68. Futagami S, Itoh T, Sakamoto C. Systematic review with meta-analysis: post-infectious functional dyspepsia. Aliment Pharmacol Ther. 2015;41(2):177–188.

69. Zhang Y, Hoffmeister M, Weck MN, Chang-Claude J, Brenner H. Helicobacter pylori infection and colorectal cancer risk: evidence from a large population-based case-control study in Germany. Am J Epidemiol. 2012;175(5):441–450.

70. Kim TJ, Kim ER, Chang DK, Kim YH, Baek SY, Kim K, et al. Helicobacter pylori infection is an independent risk factor of early and advanced colorectal neoplasm. Helicobacter. 2017 Jun;22(3). doi: 10.1111/hel.12377.

71. Franchini M, Vescovi PP, Garofano M, Veneri D. Helicobacter pylori-associated idiopathic thrombocytopenic purpura: a narrative review. Semin Thromb Hemost. 2012;38(5):463–468.

72. Cardenas VM, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and Helicobacter pylori infection in the United States. Am J Epidemiol. 2006;163(2):127–134.

73. Nam SY, Ryu KH, Park BJ, Park S. Effects of Helicobacter pylori infection and its eradication on lipid profiles and cardiovascular diseases. Helicobacter. 2015;20(2):125–132.

74. Suwarnalata G, Tan AH, Isa H, Gudimella R, Anwar A, Loke MF, et al. Augmentation of Autoantibodies by Helicobacter pylori in Parkinson's Disease Patients May Be Linked to Greater Severity. Brüggemann H, editor. PLoS One. 2016 Apr 21;11(4):e0153725. doi: 10.1371/journal.pone.0153725.

75. Wang F, Liu J, Lv Z. Association of Helicobacter pylori infection with diabetes mellitus and diabetic nephropathy: a meta-analysis of 39 studies involving more than 20,000 participants. Scand J Infect Dis. 2013;45(12):930–938.

76. Magen E, Schlesinger M, Hadari I. Chronic urticaria can be triggered by eradication of Helicobacter pylori. Helicobacter. 2013 Feb;18(1):83–87.

77. Kountouras J, Boziki M, Gavalas E, Zavos C, Deretzi G, Chatzigeorgiou S, et al. Five-year survival after Helicobacter pylori eradication in Alzheimer disease patients. Cogn Behav Neurol. 2010;23(3):199–204.

78. Kikuchi T, Kobayashi T, Yamashita T, Ohashi K, Sakamaki H, Akiyama H. Eight-year follow-up of patients with immune thrombocytopenic purpura related to H. pylori infection. Platelets. 2011;22(1):61-64.

79. Elliott DRF, Walker AW, O'Donovan M, Parkhill J, Fitzgerald RC. A non-endoscopic device to sample the oesophageal microbiota: a case-control study. Lancet Gastroenterol Hepatol.

2017;2(1):32–42.

80. Blaser MJ. Who are we? Indigenous microbes and the ecology of human diseases. EMBO Rep. 2006;7(10):956–960.

81. Blaser MJ. Disappearing microbiota: Helicobacter pylori protection against esophageal adenocarcinoma. Cancer Prev Res (Phila). 2008;1(5):308–311.

82. Whiteman DC, Parmar P, Fahey P, Moore SP, Stark M, Zhao ZZ, et al. Association of Helicobacter pylori infection with reduced risk for esophageal cancer is independent of environmental and genetic modifiers. Gastroenterology. 2010;139(1):73-83.

83. Zhou X, Wu J, Zhang G. Association between Helicobacter pylori and asthma: a meta-analysis. Eur J Gastroenterol Hepatol. 2013;25(4):460–468.

84. Vo HD, Goli S, Gill R, Anderson V, Stefanov DG, Xu J, et al. Inverse correlation between Helicobacter pylori colonization and obesity in a cohort of inner city children. Helicobacter. 2015;20(1):64–68.

85. Walker MM, Talley NJ. Review article: bacteria and pathogenesis of disease in the upper gastrointestinal tract - beyond the era of Helicobacter pylori. Aliment Pharmacol Ther. 2014;39(8):767–779.