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Gastric Microbiota: Between Health and Disease

Hristo Ilianov Iliev, Mila Dimitrova Kovacheva-Slavova, Todor Asenov Angelov, Hristo Yankov Valkov, Ali Bedran and Borislav Georgiev Vladimirov

Abstract

The etiologic link between *H. pylori* infection and gastric chronic inflammation and related complications has been well established, but pathogenic pathways are still widely discussed and not sufficiently clear. The introduction of culture-independent molecular techniques has allowed better understanding of the gastric microbiota and has revealed that, when present, *H. pylori* represents the main colonizer but is part of a far more complex and dynamic microbiota than previously thought. This conceptual shift has made way for new pathogenic theories, focused on the interrelations between *H. pylori* and other gastric microbiota. Main factors that affect the gastric microbiota are gastric acidity, inflammation, and environmental factors, such as diet and drugs. Previous studies have made progress in explaining the complex interactions between gastric microorganisms in healthy individuals and their role in the development of related gastroduodenal (peptic ulcers and gastric cancer (GC)) and extraintestinal diseases, but more scientific proof is needed. This review presents current knowledge on gastric microbiota and its role in health and in the development of gastroduodenal diseases.

Keywords: gastric microbiota, *H. pylori*, pathogenesis, chronic inflammation, peptic ulcers, gastric cancer

1. Introduction

The first isolation of *Helicobacter pylori* (*H. pylori*) in 1982 [1] changed the traditional misconception that the stomach, with its naturally hostile environment, cannot be a reservoir for microbial species. Since then *H. pylori* has been well established as a key factor in gastric pathology, being the main cause of chronic active gastritis. This inflammation can progress to often interlinked severe complications such as atrophic gastritis, peptic ulcer disease, and gastric malignancies [2]. The assumption of the uniqueness of *H. pylori* as the only bacteria able to survive in the gastric hostile environment has also fast been shattered with the advances in culture-dependent and culture-independent techniques that revealed that *H. pylori* is part of a complex gastric microbiota. The gastric microbial density is now estimated at around 10^2 to 10^4 colony-forming units (CFU)/mL [3], with variations related to local pH, site of isolation, and environmental factors, such as food ingestion and medication intake. Furthermore, the pool of species and subspecies varies related to *H. pylori* status and related pathologic complications. Due to this multitude of factors, the differentiation

between resident and transient microbiota and its role in health and disease remains controversial. Nevertheless, the potential of understanding the structure and dynamics of the gastric microbiota for the pathogenesis, diagnosis, and treatment of gastroduodenal diseases remains. Hence, this review aims to underline current knowledge on gastric microbiota and its relation to gastroduodenal pathology.

2. The hostile gastric environment

Compared to other gastrointestinal (GI) segments, the stomach has a physiological environment that is significantly more hostile to bacterial colonization and is a crucial part of the dynamics of the gastric microbiota. Primary reason for this is the gastric juice, which is composed of two main components—proteolytic enzymes and hydrochloric acid (HCl). The hydrochloric acid creates a strong acidic environment by maintaining a pH of 1–2 in the gastric lumen, which together with the proteolytic features of the gastric enzymes creates an intragastric environment that serves both digestive and protective roles. This environment facilitates the denaturation of proteins and nutrient absorption but also severely limits bacterial colonization and survival, preventing infection by pathogens [4]. The low pH value is the main restrictive component of the gastric juice [5]. To prevent damage to the mucosa from the acid and enzymes, neck cells of the gastric glands secrete mucus on the surface of the gastric epithelium. This mucus layer establishes a pH gradient that increases the pH up to 6–7 at the surface of the mucosa [6]. This is due to the unique properties of the mucus which permit acid to flow from parietal cells into crypts which communicate with the lumen, but do not allow acid at pH <4 from penetrating the mucus layer [6]. The mucus layer consists of several different mucin molecules, including MUC1, MUC5AC, MUC5AB, and MUC6, and forms two sublayers, an inner mucus layer that is firmly attached to the epithelia and a loose mucus layer, which is in direct contact with the lumen [7, 8]. Additional factors that contribute to the strong antimicrobial environment of the stomach are the accidental bile reflux and the gastric peristalsis.

3. *H. pylori*

In 1982 *Helicobacter pylori* was isolated by Barry Marshall and Robin Warren [1]. Their research changed the long-standing view that the stomach is as a sterile organ, being naturally hostile to bacterial survival. Today, it is known that *H. pylori* infects more than 50% of the world's population with the only significant reservoir for the infection appearing to be humans. Possible routes of infection include oral-oral, fecal-oral, and iatrogenic spread (e.g., by unsterile endoscopic interventions). In developing countries, infection is usually acquired early in childhood, unlike in industrialized countries, where it develops more commonly in adulthood [9].

H. pylori is a Gram-negative, spiral-shaped, motile, and flagellated bacteria that is uniquely adapted to colonizing the gastric niche. Hence, it comes with no surprise that when present, *H. pylori* has the highest relative abundance among all gastric microbial communities in both adults and children [10–12]. Upon infection, *H. pylori* utilizes urease and α -carbonic anhydrase to generate ammonia and HCO_3^- . This neutralizes H^+ and locally increases the pH, facilitating the bacteria's passage through the acidic gastric fluid and the pH-sensitive mucous layer. Using chemotaxis, the bacteria navigates the pH gradient to their niche near the host's epithelium [13, 14]. Once established in the inner mucus layer, *H. pylori* can utilize diverse adhesins (e.g., SabA and BabA) to attach to epithelial cells. Once attached, bacterial

effector molecules, such as the vacuolating cytotoxin (VacA) and the cytotoxin-associated gene A (CagA), modulate the gastric epithelial cell behavior, leading to loss of cell polarity, release of nutrients and chemokines, and regulation of acid secretion via control of gastrin and H⁺/K⁺ ATPase [9, 15, 16]. In response to the *H. pylori* infection, the host mounts a complex inflammatory response, which ultimately leads to active chronic gastritis and subsequent gastroduodenal diseases. Therefore, the host's attempts to eradicate *H. pylori* increase gastric immunopathology (gastritis, epithelial damage such as atrophy and intestinal metaplasia), which alters the gastric compartment and its microbiota and may subsequently progress to gastric cancer.

4. Other non-*H. pylori* microbiota

4.1 Culture-dependent identification of gastric microbiota

Initial studies on the bacteria present in the stomach, using culture-based techniques, such as gastric juice cultures and mucosal biopsies were reported even before the isolation of *H. pylori*. In 1977 Savage DC [17] isolated bacteria from the stomach estimated at $>10^3$ CFU/g. The predominant phyla were *Firmicutes* (genera *Lactobacillus*, *Streptococcus*, *Clostridium*, and *Veillonella*), *Actinobacteria* (genus *Bifidobacterium*), and *Proteobacteria* (coliforms). However, due to the fact that these bacteria are prevalent along the whole GI tract, they were considered transient bacteria, which form small colonies that exist for short periods of time, rather than true gastric colonizers. Later culture-based studies [18–23] find that the most prevalent phylum, regardless of *H. pylori* status, is *Firmicutes*, followed by *Proteobacteria* and *Bacteroidetes*. *Actinobacteria* varies in studies as the second or third most prevalent phylum. The most commonly found genera were *Streptococcus*, *Lactobacillus*, *Bacteroides*, *Staphylococcus*, *Veillonella*, *Corynebacterium*, and *Neisseria*. However, given that culturing conditions for the majority of microbes colonizing the GI tract are not established, culture-based methods are considered to underestimate the gastric microbial diversity and are largely replaced by culture-independent methods.

4.2 Culture-independent identification of gastric microbiota

Culture-independent studies use a variety of molecular methods based on 16S rRNA gene sequencing. A multitude of reasons define these methods as far superior to those which are culture-dependent. These include:

- 16S rRNA is present in almost all bacteria.
- The function of the 16S rRNA gene has remained unchanged over time, suggesting that random sequence changes are a more accurate measure of time (evolution).
- The 16S rRNA gene is large enough for computational purposes [24].

A variety of 16S rRNA based methods exist, including:

- Fluorescent in situ hybridization (FISH) [25]
- Dot-blot hybridization with rRNA-targeted probes [26]
- Targeted qPCR [27]

- Traditional or sequence-aided community fingerprinting [28]
- Temperature gradient gel electrophoresis (TGGE) [29]
- Terminal restriction fragment length polymorphism (T-RFLP) [30]
- Sequencing of cloned 16S rDNA [29]
- Microarrays (PhyloChip) [31]
- Next-generation sequencing (NGS) [32]

In an extensive review of eight culture-independent studies, Sheh A. and Fox J. concluded that the most prominent phyla in the stomach are *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria* [33]. Furthermore, the most abundant phyla in *H. pylori*-positive subjects are *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. In the absence of *H. pylori*, the most abundant phyla are *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. However, *H. pylori* remains the most dominant species in the stomach, comprising 72–99% of sequencing reads [10, 34]. In the absence of *H. pylori*, analysis consistently shows the presence of *Streptococcus* spp., which seem to be the most abundant genus in *H. pylori*-negative subjects [3, 30, 35, 36]. In the gastric microbiota, the non-*Helicobacter* genera commonly detected are *Streptococcus*, *Prevotella*, *Veillonella*, and *Rothia*.

5. Interrelations between *H. pylori* and other gastric microbiota

Numerous studies have shown significant variability of the gastric microbial communities with respect to *H. pylori* status. One such study was carried out by Osaki et al. [37], who examined the gastric microbiota of *H. pylori*-positive and *H. pylori*-negative Mongolian gerbils. The study showed a larger number of *Bifidobacterium* spp. in *H. pylori*-positive gerbils, compared to the *H. pylori*-negative, while *Eubacterium cylindroides* and *Prevotella* spp. were only found in the *H. pylori*-negative group.

Several mouse model studies have also shown clear differences in the composition of the gastric microbiota with respect to *H. pylori* status. Infection by *H. pylori* of pathogen-free female BALB/c mice has been shown to reduce the *Lactobacillus* spp. in the gastric microflora [26]. In transgenic, insulin-gastrin (INS-GAS) mice, the *H. pylori*-infected male mice show a significantly different phyla compared to the non-infected control group, with an increase in *Firmicutes* and a decrease in *Bacteroidetes* [26]. Findings in *H. pylori*-colonized C57BL/6 N female mice included reductions in *Firmicutes* (class *Bacilli*), *Bacteroidetes*, and *Proteobacteria* and an increase of *Firmicutes* (class *Clostridia*), *Proteobacteria* (genus *Helicobacter*), and *Verrucomicrobia*. However, other published data on the murine gastric microbiota suggest that neither acute nor chronic *H. pylori* infection substantially modifies the gastric microbial ecosystem [38].

A few studies have also examined *H. pylori*-related microbial differences in humans. One study found relative abundances of *Proteobacteria*, *Spirochetes*, and *Acidobacteria* in *H. pylori*-positive patients, compared to the control *H. pylori*-negative group. Another study demonstrated that patients positive for *H. pylori* culture showed significantly increased colonization of *Proteobacteria* and a decrease in *Actinobacteria* [39].

A few studies have given insight on how other microbial species can affect *H. pylori* by modulating *H. pylori*-induced gastric inflammatory responses. Two studies have

indicated that the presence of intestinal *Helicobacter* (*H. bilis*, *H. hepaticus*, and *H. muridarum*) can both increase and decrease the severity of *H. pylori*-induced gastric inflammation by altering Treg cell responses [40, 41]. Another study demonstrated that *H. pylori* is present within the intestine in a coccoid form and that its interaction with phagocytes within the intestinal Peyer's patches modifies the intensity of *H. pylori*-induced gastritis [42]. However, other studies have shown that the gastric microbiota can accelerate gastric cancer progression in the presence of *H. pylori* and does so with no differences detected in the composition of the intestinal microbiota [27, 32].

As previously described *H. pylori* infection is associated with a multitude of changes in the gastric physiology and immunology, e.g., reduced gastric acidity, disturbed nutrient availability, and local inflammatory responses. These changes might be one explanation for the shift in the gastric microbial communities described, but the relation between *H. pylori* and non-*H. pylori* microbiota seems to be far more complex and remains to be further clarified. One problem is that so far, most studies are focused only on the effects of *H. pylori* on other microbiota, but little is known of how *H. pylori* is affected by other resident bacteria. Another problem is that different studies cannot be objectively compared, since they highly vary in methods and models used and the results depend on numerous other factors such as the time of *H. pylori* infection and the degree of mucosal inflammation. Therefore, further experiments are needed to give a more extensive understanding of these complex microbial interrelations.

6. Factors affecting the gastric microbiota

As described, *H. pylori* is the most significant species that colonizes the stomach and a key factor for the gastric microbial diversity but is far from sufficient to provide a wholesome understanding of the factors that determine its dynamics. Major factors that influence and define the dynamics of the gastric microbiota include gastric acidity, inflammation of the gastric mucosa, dietary habits, and use of medications.

6.1 Gastric acidity

The human gastric juice has an interprandial pH of between 1 and 2 in the gastric lumen, whereas with food ingestion it can reach up to pH 5. pH also varies in the different anatomical regions of the stomach, with most acidic being the fundus and the least being the antrum. The mucus lining the gastric mucosa establishes also a pH gradient from the lumen to the surface of the epithelium. This mucus consists of two sublayers—an inner mucus layer that is firmly attached to the epithelium and a variable mucus layer directly interacting with the lumen [7, 8]. Thus, across the mucus layer, the pH ranges from about 5.5 to 6.8 or even 7 at the surface of the gastric epithelial cells [5, 6]. It was already discussed that the low pH, caused by the hydrochloric acid, restricts the quantity of microorganisms and reduces the risk of infection by pathogens. Hence, sites with higher pH are significantly more hospitable to colonization and have a higher microbial density. Considerable fluctuations in the microbial density have been described with respect to the pH in the stomach, whereby both the quantity and the proportion of genera also fluctuate [43, 44]. Bacteria and bacterial DNA, which are isolated from gastric juice, differ from bacterial isolates adhering to the mucosa. During abnormal conditions, this balance may be different.

6.2 Dietary habits

While many studies document the effects of diet on the gut microbiota composition in humans, [45–49] there are only a few, mainly animal model studies,

addressing the influence of diet on the gastric microbiota. An example is an in vivo study that compared the gastric microbiota of mice fed a non-purified diet (natural source-derived food) to mice fed a purified diet (refined food) and found higher levels of total aerobes, total anaerobes, and *Lactobacillus* in the stomach of the mice on a non-purified diet [50]. Nevertheless, it is well established how dietary factors affect the gastric microclimate, and since the microbiota is an inseparable part of this microclimate, it is not farfetched to suspect that diet affects the gastric microbial communities. However, more research is needed.

6.3 Use of medications

The long-term use of proton pump inhibitors (PPIs) and H₂ antagonists affects the composition of the gastric microbiota by inducing a non-*H. pylori* bacterial overgrowth [51]. This is not surprising, considering that normally the non-*H. pylori* gastric microbiota is suppressed by the significantly acidic gastric environment. Suppression of gastric acidity will alter the bacterial flora of the upper GI tract, and studies have confirmed that PPIs do alter the bacterial population in the stomach [52]. This is mainly due to oral bacteria that survive instead of being killed in the normally acidic stomach. It has also been suspected that by causing alteration and overgrowth of the microbiota, acid-suppressive treatments may increase the risk of gastric cancer [52]. It has also been shown that a previously antrum-dominant *H. pylori* infection after treatment with acid inhibitors changed to a more corpus predominant infection [51]. The less acidic corpus allows *H. pylori* to penetrate deeper in the crypts and increase the inflammation, which causes faster progression to atrophy [53]. Treatment with acid inhibitors has by culture-dependent methods been shown to affect the survival of bacteria in the stomach. However, no significant differences have been found regarding diversity and composition of the microbiota by using culture methods [10].

Antibiotics are well known to have suppressive effects on the gastrointestinal microflora. *H. pylori* eradication is dependent on combined antibiotic treatment. However, certain antibiotic treatments can have negative effects on the “healthy” gastric microbiota. Animal studies indicate that treatment with penicillins reduces *Lactobacillus* populations and promotes yeast colonization of the gastric epithelium. Furthermore, Mason et al. [54] showed that cefoperazone treatment in humans causes long-term alteration of the gastric microbiota, such as a significant reduction in the number of *Lactobacillus* and overgrowth of *Enterococcus*.

7. Gastric microbiota and gastroduodenal diseases

7.1 Chronic gastritis and peptic ulcer disease

The isolation of *H. pylori* was a real breakthrough, not because it declared the stomach as a non-sterile organ but because subsequent research established it as a main etiological factor for the development of gastroduodenal diseases [2, 55, 56]. It is well known that long-term *H. pylori* infection causes various degrees of chronic inflammation of the underlying gastric mucosa. A subset of patients develops clinical symptoms, and a further subset will develop complications including peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. The cascade leading from chronic gastritis to neoplasm is known as Correa’s cascade and involves the progression to glandular atrophy with intestinal metaplasia and dysplasia and eventually to invasive carcinoma [57]. Peptic ulcer disease is the most common complication of chronic *H. pylori* infection, with 95% of duodenal and 70% of gastric ulcers being linked to it [58]. All of this serves to prove that *H. pylori*

is by far the most important microbial species that can colonize the stomach, with an enormous impact on the pathogenesis and development of gastroduodenal diseases.

Nevertheless, there is arising evidence that non-*H. pylori* bacteria may also play an important role in the pathogenesis of chronic gastritis and peptic ulcers. One study suggests that different gastric microbial communities, such as the overrepresentation of the *Streptococcus* genus within the *Firmicutes* phylum, can lead to gastritis as well, even in the absence of *H. pylori* [35]. Another study has found an increase of *Streptococcus* and a decrease of *Prevotella* in patients with atrophic gastritis, versus healthy subjects [59]. There are also several studies that assess the role of non-*H. pylori* species in peptic ulcer disease. The non-*H. pylori* bacteria seems to be more prevalent in patients with non-ulcer dyspepsia than in those with gastric ulcer as shown by a culture-based study by Hu et al. [19]. Another study in Malaysian patients showed significant correlation between the isolation of *Streptococcus* and patients with peptic ulcers [60]. Given that most studies find that the *Streptococcus* genus is one of the most abundant non-*H. pylori* species in the stomach, a possible pathogenic relation can be a subject of further research. However, by far no other species have an established pathogenic role except *H. pylori*.

7.2 Gastric cancer

Each year approximately 990,000 people are diagnosed with gastric cancer (GC) worldwide, of whom about 738,000 die from this disease, making GC the fourth most common incident cancer and the second most common cause of cancer death. Both incidence and mortality rates are about twice as high in males as in females. Over 70% of cases occur in developing nations, concentrated in Eastern Asia, Eastern Europe, and Central and South America. Approximately 90% of gastric cancers are adenocarcinomas, with the other 10% shared between mucosa-associated lymphoid tissue (MALT) lymphomas, gastrointestinal stromal tumors (GIST), leiomyosarcomas, and other more rare types of cancer. Adenocarcinomas are histologically classified into two major types: diffuse and intestinal. These two types not only look different under the microscope but also differ in gender ratio, age at diagnosis, and other epidemiologic features. Anatomically, gastric cancers are categorized as proximal and distal. Proximal adenocarcinomas are more similar to esophageal adenocarcinomas and may be associated with the absence of *H. pylori*, while distal adenocarcinomas originate in the antrum, with approximately 90% of such cases related to *H. pylori* infection [61]. Today the correlation between *H. pylori* and the development of gastric cancer is undeniable as shown in several prospective studies [62–64]. Moreover, the eradication of *H. pylori* is proven to significantly reduce the risk of gastric cancer development, according to several international consensus [65, 66]. *H. pylori* was recognized as a “definite carcinogen” by the World Health Organization in 1994, and this fact was reconfirmed in 2009.

However, *H. pylori* coevolved with humans for millennia, and only 1–2% of people infected with this bacterium actually develop gastric cancer or MALT lymphoma. Similar to most cancers, pathogenetic mechanisms remain unclear with a multitude of other factors to influence the final carcinogenesis [61]. Although, *H. pylori* is clearly the most relevant microbial risk factor for the development of gastric cancer, an increasing pool of evidence suggests that other microbial communities play a causative role in the pathophysiology of gastric cancer. To date, several animal and human studies have supported this theory.

Studies with INS-GAS mice have revealed that male mice with intestinal microbiota developed gastric pathology from chronic gastritis to atrophy and dysplasia independent of *H. pylori* infection. Furthermore, the presence of commensal microbiota accelerated the progression to gastric intraepithelial neoplasia, and gastric intraepithelial neoplasia became invasive in *H. pylori*-infected INS-GAS mice. Male INS-GAS mice

with *H. pylori* infection, colonized with artificial mouse intestinal microbiota, have shown increased incidence of gastric intraepithelial neoplasia by 69% [27, 32]. On the other hand, antibiotic treatments significantly delayed the onset of gastric neoplasia in *Helicobacter*-free and specific pathogen-free INS-GAS mice [67].

A study by Wang et al. found a similar number of bacterial species in the microbiota between gastric cancer and chronic gastritis, but by using a method to explore and visualize similarities or dissimilarities of the data, a pattern suggesting the presence of a diversified microbiota in gastric cancer was found [68]. Moreover, a 16S rRNA gene sequencing analysis of gastric mucosa of patients with gastric cancer showed a prevalence of the genera *Lactobacillus*, *Streptococcus* (among which the most common species were *S. mitis* and *Streptococcus parasanguinis*), *Prevotella*, and *Veillonella* [30]. Two other studies evaluated the gastric microbiota of subjects with non-atrophic gastritis, intestinal metaplasia, and gastric cancer. The first one showed a significantly lower diversity and a higher abundance of the genus *Pseudomonas* in the microbiota of neoplastic patients compared to patients with simple gastritis. Moreover, both the progressive decrease of six taxa and the progressive increase of two taxa were observed from the gastritis group to the neoplastic group, via the metaplastic group [69]. In the second study, a high-throughput sequencing platform was used for the assessment of gastric microbiota in the three groups, showing definitely different results: a greater bacterial diversity, a relative rise of *Bacilli* and *Streptococcaceae*, and a relative reduction of *Helicobacteraceae* were found in the cancer group compared to other groups [70].

It is possible that non-*H. pylori* species potentiate carcinogenesis through various mechanisms, such as promoting inflammation, stimulating cell proliferation, modifying stem cell dynamics, and producing toxic metabolites [71]. However, it is still unsure whether the different microbial structure is causative for the carcinogenesis or carcinogenesis itself causes a shift in the microbial communities, which subsequently promotes carcinogenesis further.

8. Gastric microbiota and extra-gastric diseases

The stomach is part of the GI tract, and as such, possible relations between the gastric microbiota's composition and diseases of other parts of the GI tract, such as the esophagus (esophagitis and esophageal cancer), small intestines, and colon cannot be overlooked [72]. One study, using 16S rDNA analyses of duodenal aspirates, demonstrated lower diversity in irritable bowel syndrome patients compared to controls with significant alterations in 12 genera [73]. An increased risk for colorectal neoplasia in *H. pylori*-infected patients has been confirmed by many large-scale studies [74, 75].

Other studies have addressed the association between autoimmune hepatitis and altered microbiome of the upper GI tract and found this to be linked to increased intestinal permeability [76].

Regarding the extra-gastrointestinal involvement of gastric microbiota (especially *H. pylori*), studies find possible associations with hematological diseases like idiopathic thrombocytopenic purpura [77] and anemia [78] and cardiovascular [79], neurological [80], and endocrine [81] diseases.

Nevertheless, research in this field is far from sufficient to be conclusive.

9. Discussion

It is undeniable that *H. pylori* is by far the most unique and important species that can colonize the gastric niche with clear pathogenic significance to

gastroduodenal diseases. However, culture-dependent and later culture-independent studies prove that the stomach harbors a complex microbiota with many other phyla (*Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*) and genera (*Lactobacillus*, *Streptococcus*, *Clostridium*, *Prevotella*, *Veillonella*, *Bifidobacterium*, and *Rothia*) being identified. By far, it is arguable which genera can be considered resident since results depend on a multitude of factors, among which gastric acidity stands out as the most significant. However, it is worth noting that *Streptococcus*, *Prevotella*, *Veillonella*, and *Rothia* seem to be the most abundant genera in *H. pylori*-negative subjects. Nevertheless, the significance of both transient and resident non-*H. pylori* microbiota lies in its possible role in the development and progression of gastroduodenal diseases, such as gastritis, peptic ulcer disease, and gastric cancer. So far, only a few and far from enough studies suggest that non-*H. pylori* microbiota can lead to gastric pathology in the absence of *H. pylori*. However, findings are convincing that the non-*H. pylori* microbiota can play an important role in modulating *H. pylori*-induced gastric inflammation, influencing an individual's risk of gastric diseases and consequently the severity of the resulting disease. Suspected pathophysiologic mechanisms involved in this include modulating immune cell responses, stimulating cell proliferation, modifying stem cell dynamics, and producing toxic metabolites. Although, substantial advancements in unveiling the complexity of the gastric microbiota and its' role in health and disease have been made, studies are far from sufficient to suggest new strategies for prevention, diagnosis, and treatment of gastroduodenal diseases. However, this potential remains and undoubtedly should be further explored.

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Conflict of interest

There are no conflicts of interest.

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References

- [1] Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*. 1983;**1**:1273-1275. DOI: 10.1016/S0140-6736(83)92719-8
- [2] Wang F, Meng W, Wang B, Qiao L. *Helicobacter pylori*-induced gastric inflammation and gastric cancer. *Cancer Letters*. 2014;**345**(2):196-202. DOI: 10.1016/j.canlet.2013.08.016
- [3] Delgado S, Cabrera-Rubio R, Mira A, Suárez A, Mayo B. Microbiological survey of the human gastric ecosystem using culturing and pyrosequencing methods. *Microbial Ecology*. 2013;**65**(3):763-772. DOI: 10.1007/s00248-013-0192-5
- [4] Martinsen TC, Bergh K, Waldum HL. Gastric juice: A barrier against infectious diseases. *Basic & Clinical Pharmacology & Toxicology*. 2005;**96**:94-102. DOI: 10.1111/j.1742-7843.2005.pto960202.x
- [5] Manson JM, Rauch M, Gilmore MS. The commensal microbiology of the gastrointestinal tract. *Advances in Experimental Medicine and Biology*. 2008;**635**:15-28. DOI: 10.1007/978-0-387-09550-9_2
- [6] Bhaskar KR, Garik P, Turner BS, Bradley JD, Bansil R, Stanley HE, et al. Viscous fingering of HCl through gastric mucin. *Nature*. 1992;**360**:458-461. DOI: 10.1038/360458a0
- [7] Corfield AP, Carroll D, Myerscough N, Probert CS. Mucins in the gastrointestinal tract in health and disease. *Frontiers in Bioscience*. 2001;**6**:D1321-D1357. DOI: 10.2741/Corfield
- [8] Atuma C, Strugala V, Allen A, Holm L. The adherent gastrointestinal mucus gel layer: Thickness and physical state in vivo. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2001;**280**:G922-G929. DOI: 10.1152/ajpgi.2001.280.5.G922
- [9] Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clinical Microbiology Reviews*. 2006;**19**(3):449-490. DOI: 10.1128/CMR.00054-05
- [10] Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**(3):732-737. DOI: 10.1073/pnas.0506655103
- [11] Schulz C, Schütte K, Koch N, et al. The active bacterial assemblages of the upper GI tract in individuals with and without *Helicobacter* infection. *Gut*. 2016;**67**(2):216-225. DOI: 10.1136/gutjnl-2016-312904
- [12] Llorca L, Pérez-Pérez G, Urruzuno P, et al. Characterization of the gastric microbiota in a pediatric population according to *Helicobacter pylori* status. *The Pediatric Infectious Disease Journal*. 2017;**36**(2):173-178. DOI: 10.1097/INF.0000000000001383
- [13] Williams SM, Chen YT, Andermann TM, Carter JE, McGee DJ, Ottemann KM. *Helicobacter pylori* chemotaxis modulates inflammation and bacterium-gastric epithelium interactions in infected mice. *Infection and Immunity*. 2007;**75**:3747-3757. DOI: 10.1128/IAI.00082-07
- [14] Croxen MA, Sisson G, Melano R, Hoffman PS. The *Helicobacter pylori* chemotaxis receptor TlpB (HP0103) is required for pH taxis and for colonization of the gastric mucosa. *Journal of Bacteriology*. 2006;**188**:2656-2665. DOI: 10.1128/JB.188.7.2656-2665.2006

- [15] Saha A, Backert S, Hammond CE, Gooz M, Smolka AJ. *Helicobacter pylori* CagL activates ADAM17 to induce repression of the gastric H, K-ATPase alpha subunit. *Gastroenterology*. 2010;**139**:239-248. DOI: 10.1053/j.gastro.2010.03.036
- [16] O’Keeffe J, Moran AP. Conventional, regulatory, and unconventional T cells in the immunologic response to *Helicobacter pylori*. *Helicobacter*. 2008;**13**:1-19. DOI: 10.1111/j.1523-5378.2008.00559.x
- [17] Savage DC. Microbial ecology of the gastrointestinal tract. *Annual Review of Microbiology*. 1977;**31**:107-133. DOI: 10.1146/annurev.mi.31.100177.000543
- [18] Stockbruegger RW. Bacterial overgrowth as a consequence of reduced gastric acidity. *Scandinavian Journal of Gastroenterology. Supplement*. 1985;**111**:7-16. DOI: 10.3109/00365528509093749
- [19] Hu Y, He LH, Xiao D, Liu GD, Gu YX, Tao XX, et al. Bacterial flora concurrent with *Helicobacter pylori* in the stomach of patients with upper gastrointestinal diseases. *World Journal of Gastroenterology*. 2012;**18**:1257-1261. DOI: 10.3748/wjg.v18.i11.1257
- [20] Zilberstein B, Quintanilha AG, Santos MA, Pajacki D, Moura EG, Alves PR, et al. *Clinics (São Paulo, Brazil)*. 2007;**62**:47-54. DOI: 10.1590/S1807-59322007000100008
- [21] Sharma BK, Santana IA, Wood EC, Walt RP, Pereira M, Noone P, et al. Intra-gastric bacterial activity and nitrosation before, during, and after treatment with omeprazole. *British Medical Journal*. 1984;**289**:717-719. DOI: 10.1136/bmj.289.6447.717
- [22] Adamsson I, Nord CE, Lundquist P, Sjöstedt S, Edlund C. Comparative effects of omeprazole, amoxycillin plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter pylori*-infected patients. *The Journal of Antimicrobial Chemotherapy*. 1999;**44**:629-640. DOI: 10.1093/jac/44.5.629
- [23] Sjöstedt S, Heimdahl A, Kager L, Nord CE. Microbial colonization of the oropharynx, esophagus and stomach in patients with gastric diseases. *European Journal of Clinical Microbiology*. 1985;**4**:49-51. DOI: 10.1007/BF02148660
- [24] Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*. 2007;**45**(9):2761-2764. DOI: 10.1128/JCM.01228-07
- [25] Langendijk PS, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MH, et al. Quantitative fluorescence in situ hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA targeted probes and its application in fecal samples. *Applied and Environmental Microbiology*. 1995;**61**:3069-3075
- [26] Aebischer T, Fischer A, Walduck A, Schlötelburg C, Lindig M, Schreiber S, et al. Vaccination prevents *Helicobacter pylori*-induced alterations of the gastric flora in mice. *FEMS Immunology and Medical Microbiology*. 2006;**46**: 221-229. DOI: 10.1111/rp10.1016-j.femsim.2004.05.008
- [27] Lertpiriyapong K, Whary MT, Muthupalani S, Lofgren JL, Gamazon ER, Feng Y, et al. Gastric colonisation with a restricted commensal microbiota replicates the promotion of neoplastic lesions by diverse intestinal microbiota in the *Helicobacter pylori* INS-GAS mouse model of gastric carcinogenesis. *Gut*. 2013;**63**:54-63. DOI: 10.1136/gutjnl-2013-305178
- [28] Heilig HG, Zoetendal EG, Vaughan EE, Marteau P, Akkermans AD,

de Vos WM. Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Applied and Environmental Microbiology*. 2002;**68**:114-123. DOI: 10.1128/AEM.68.1.114-123.2002

[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 16SrDNA sequence analysis. *Journal of Medical Microbiology*. 2000;**49**:817-822. DOI: 10.1099/0022-1317-49-9-817

[30] Dicksved J, Lindberg M, Rosenquist M, Enroth H, Jansson JK, Engstrand L. Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. *Journal of Medical Microbiology*. 2009;**58**:509-516. DOI: 10.1099/jmm.0.007302-0

[31] Rolig AS, Cech C, Ahler E, Carter JE, Ottemann KM. The degree of *Helicobacter pylori*-triggered inflammation is manipulated by preinfection host microbiota. *Infection and Immunity*. 2013;**81**:1382-1389. DOI: 10.1128/IAI.00044-13

[32] Lofgren JL, Whary MT, Ge Z, Muthupalani S, et al. Lack of commensal flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology*. 2011;**140**:210-220. DOI: 10.1053/j.gastro.2010.09.048

[33] Sheh A, Fox JG. The role of the gastrointestinal microbiome in *Helicobacter pylori* pathogenesis. *Gut Microbes*. 2013;**4**(6):505-531. DOI: 10.4161/gmic.26205

[34] Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis

of human gut microbiota by barcoded pyrosequencing. *PLoS One*. 2008;**3**:e2836. DOI: 10.1371/journal.pone.0002836

[35] 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;**4**:e7985. DOI: 10.1371/journal.pone.0007985

[36] Stearns JC, Lynch MD, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, et al. Bacterial biogeography of the human digestive tract. *Scientific Reports*. 2011;**1**:170. DOI: 10.1038/srep00170

[37] 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*. *Microbial Pathogenesis*. 2012;**53**(1):12-18

[38] Tan MP, Kaparakis M, Galic M, Pedersen J, Pearse M, Wijburg OL, et al. Chronic *Helicobacter pylori* infection does not significantly alter the microbiota of the murine stomach. *Applied and Environmental Microbiology*. 2007;**73**(3):1010-1013

[39] Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, et al. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. *The ISME Journal*. 2010;**5**(4):574-579. DOI: 10.1038/ismej.2010.149

[40] Lemke LB, Ge Z, Whary MT, et al. Concurrent *Helicobacter bilis* infection in C57BL/6 mice attenuates proinflammatory *H. pylori*-induced gastric pathology. *Infection and Immunity*. 2009;**77**(5):2147-2158. DOI: 10.1128/IAI.01395-08

[41] Ge Z, Feng Y, Muthupalani S, et al. *Infection and Immunity*.

2011;**79**(10):3861-3871. DOI: 10.1128/IAI.05357-11

[42] Nagai S, Mimuro H, Yamada T, et al. Role of Peyer's patches in the induction of *Helicobacter pylori*-induced gastritis. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(21):8971-8976. DOI: 10.1073/pnas.0609014104

[43] Draser BS, Shiner M, McLeod GM. Studies of the intestinal flora 1. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric patients. Gastroenterology. 1969;**56**:71-79

[44] Milton-Thompson GJ, Lightfoot NF, Ahmet Z, et al. Intra-gastric acidity, bacteria, nitrite, and N-nitroso compounds before, during, and after cimetidine treatment. Lancet. 1982;**1**(8281):1091-1095

[45] Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: Downstream impacts on host immune function and health, and therapeutic implications. Journal of Gastroenterology. 2014;**49**(5):785-798. DOI: 10.1007/s00535-014-0953-z

[46] Korpela K, Flint HJ, Johnstone AM, et al. Gut microbiota signatures predict host and microbiota responses to dietary interventions in obese individuals. PLoS One. 2014;**9**(6):e90702. DOI: 10.1371/journal.pone.0090702

[47] David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2013;**505**(7484):559-563. DOI: 10.1038/nature12820

[48] Chan YK, Estaki M, Gibson DL. Clinical consequences of diet-induced dysbiosis. Annals of Nutrition and Metabolism. 2013;**63**(suppl 2):28-40. DOI: 10.1159/000354902

[49] Fan W, Huo G, Li X, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in infants during the six months of life. Journal of Microbiology and Biotechnology. 2014;**24**(2):133-143

[50] Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;**334**:105-108. DOI: 10.1126/science.1208344

[51] Sanduleanu S, Jonkers D, De Bruine A, Hameeteman W, Stockbrügger RW. Non-*Helicobacter pylori* bacterial flora during acid-suppressive therapy: Differential findings in gastric juice and gastric mucosa. Alimentary Pharmacology & Therapeutics. 2001;**15**(3):379-388. DOI: 10.1046/j.1365-2036.2001.00888.x

[52] Williams C, McColl KEL. Review article: Proton pump inhibitors and bacterial overgrowth. Alimentary Pharmacology & Therapeutics. 2006;**23**(1):3-10. DOI: 10.1111/j.1365-2036.2006.02707.x

[53] Meuwissen SG, Craanen ME, Kuipers EJ. Gastric mucosal morphological consequences of acid suppression: A balanced view. Best practice & research. Clinical Gastroenterology. 2001;**15**(3):497-510. DOI: 10.1053/bega.2001.0189

[54] 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. Infection and Immunity. 2012;**80**(1):150-158. DOI: 10.1128/IAI.05162-11

[55] Zhang X, Zhang J, Lin Y, Xu K, Li N, Chen H, et al. Analysis of the relationship between invasive capability of *Helicobacter pylori* and gastroduodenal diseases. Journal of Medical Microbiology. 2015

May; **64**(Pt 5):498-506. DOI: 10.1099/jmm.0.000049

[56] Blaser MJ, Atherton JC. *Helicobacter pylori* persistence: Biology and disease. The Journal of Clinical Investigation. 2004; **113**(3):321-333. DOI: 10.1172/JCI20925

[57] Correa P, Piazuelo MB. *Helicobacter pylori* infection and gastric adenocarcinoma. US Gastroenterology and Hepatology Review. 2011; **7**(1):59-64

[58] Ford AC, Gurusamy KS, Delaney B, Forman D, Moayyedi P. Eradication therapy for peptic ulcer disease in *Helicobacter pylori*-positive people. Cochrane Database of Systematic Reviews. 2016; **4**:CD003840. DOI: 10.1002/14651858.CD003840.pub5

[59] Engstrand L, Lindberg M. *Helicobacter pylori* and the gastric microbiota. Best Practice & Research. Clinical Gastroenterology. 2013; **27**: 39-45. DOI: 10.1016/j.bpg.2013.03.016

[60] Khosravi Y, Dieye Y, Poh BH, et al. Culturable bacterial microbiota of the stomach of *Helicobacter pylori* positive and negative gastric disease patients. The Scientific World Journal. 2014; **2014**:610421. DOI: 10.1155/2014/610421

[61] Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: Descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiology, Biomarkers & Prevention. 2014; **23**(5):700-713. DOI: 10.1158/1055-9965.EPI-13-1057

[62] Forman D, Newell DG, Fullerton F, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: Evidence from a prospective investigation. BMJ. 1991; **302**(6788):1302-1305

[63] Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. The New England Journal of Medicine. 1991; **325**(16):1132-1136. DOI: 10.1056/NEJM199110173251604

[64] Hsu PI, Lai KH, Hsu PN, et al. *Helicobacter pylori* infection and the risk of gastric malignancy. The American Journal of Gastroenterology. 2007; **102**(4):725-730. DOI: 10.1111/j.1572-0241.2006.01109.x

[65] Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection--the Maastricht IV/ Florence consensus report. Gut. 2012; **61**(5):646-664. DOI: 10.1136/gutjnl-2012-302084

[66] Sheu BS, Wu MS, Chiu CT, 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; **22**(3):e12368. DOI: 10.1111/hel.12368

[67] Lee CW, Rickman B, Rogers AB, et al. Combination of sulindac and antimicrobial eradication of *Helicobacter pylori* prevents progression of gastric cancer in hypergastrinemic INS-GAS mice. Cancer Research. 2009; **69**(20):8166-8174. DOI: 10.1158/0008-5472.CAN-08-3856

[68] Wang L, Zhou J, Xin Y, Geng C, Tian Z, Yu X, et al. Bacterial overgrowth and diversification of microbiota in gastric cancer. European Journal of Gastroenterology & Hepatology. 2016; **28**(3):261-266. DOI: 10.1097/MEG.0000000000000542

[69] 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**:407-416. DOI: 10.1111/hel.12145
- [70] Walker MM, Talley NJ. Review article: Bacteria and pathogenesis of disease in the upper gastrointestinal tract—beyond the era of *Helicobacter pylori*. *Alimentary Pharmacology & Therapeutics*. 2014;**39**(8):767-779. DOI: 10.1111/apt.12666
- [71] Alarcon T, Lorca L, Pereze-Perez G. Impact of microbiota and gastric disease development by *Helicobacter pylori*. *Current Topics in Microbiology and Immunology*. 2017;**400**:253-275. DOI: 10.1007/978-3-319-50520-6_11
- [72] Ianiro G, Molina-Infante J, Gasbarrini A. Gastric microbiota. *Helicobacter*. 2015;**20**(Suppl. 1):68-71. DOI: 10.1111/hel.12260
- [73] Giamarellos-Bourboulis E, Tang J, Pyleris E, Pistiki A, Barbatzas C, Brown J, et al. Molecular assessment of differences in the duodenal microbiome in subjects with irritable bowel syndrome. *Scandinavian Journal of Gastroenterology*. 2015;**50**(9):1076-1087. DOI: 10.3109/00365521.2015.1027261
- [74] 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. *American Journal of Epidemiology*. 2012;**175**(5):441-450. DOI: 10.1093/aje/kwr331
- [75] Kim TJ, Kim ER, Chang DK, et al. *Helicobacter pylori* infection is an independent risk factor of early and advanced colorectal neoplasm. *Helicobacter*. 2017;**22**(3):e12377. DOI: 10.1111/hel.12377
- [76] Lin R, Zhou L, Zhang J, Wang B. Abnormal intestinal permeability and microbiota in patients with autoimmune hepatitis. *International Journal of Clinical and Experimental Pathology*. 2015;**8**(5):5153-5160
- [77] Franchini M, Vescovi PP, Garofano M, Veneri D. *Helicobacter pylori*-associated idiopathic thrombocytopenic purpura: A narrative review. *Seminars in Thrombosis and Hemostasis*. 2012;**38**(5):463-468. DOI: 10.1055/s-0032-1305781
- [78] Cardenas VM, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and *Helicobacter pylori* infection in the United States. *American Journal of Epidemiology*. 2006;**163**(2):127-134. DOI: 10.1093/aje/kwj018
- [79] Nam SY, Park BJ, Ryu KH, Nam JH. Effect of *Helicobacter pylori* eradication on the regression of gastric polyps in National Cancer Screening Program. *The Korean Journal of Internal Medicine*. 2017;**33**(3):506-511. DOI: 10.3904/kjim.2016.286
- [80] Suwarnalata G, Tan AH, Isa H, et al. Augmentation of autoantibodies by *Helicobacter pylori* in Parkinson's disease patients may be linked to greater severity. *PLoS One*. 2016;**11**(4):e0153725. DOI: 10.1371/journal.pone.0153725
- [81] 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. *Scandinavian Journal of Infectious Diseases*. 2013;**45**(12):930-938. DOI: 10.3109/00365548.2013.844351