

## Review Article

# *H. pylori* and Human Gut Microbiota

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## Abstract

The composition of the Gastrointestinal (GI) microbiome is shaped by a variety of factors including diet, additional environmental elements, and the genetic background of the host.

Recent evidence supports that *H. pylori* is the most relevant, but may not be the only local causative bacteria leading to gastric diseases. In several recent reports, a real, active cross talk between *H. pylori* and the other components of the gastric microbiota was observed. In this review, we provide a comprehensive review about gastric microbiota and discuss emerging concepts for the influence of *H. pylori* infection on gastric microbiome and *vice versa*.

## *H. pylori* Infection and Gastric Microbiota

GI microbiota had been investigated by cultivation of luminal contents or mucosal biopsies, and the human stomach was long thought to be sterile. The new nucleotide sequencing techniques and advanced bioinformatics tools have opened the field for studying the diversity and complexity of the GI microbiome independent of traditional cultural methods. Increasing number of papers focus on non-*H. pylori* microbial community observed in the human stomach, so called human gastric microbiota [1]. Recent evidence supports that *H. pylori* is the most relevant, but may not be the only local causative bacteria leading to gastric diseases. The discrepancies of the results in the previous studies are likely due to different methods used for microbiota analysis, the limited sample sizes, difference in *H. pylori* infection rates, and several environmental factors such as diet, lifestyle, geography, and ethnicity. In several recent reports, a real, active cross talk between *H. pylori* and the other components of the gastric microbiota was observed. In this review, we provide a comprehensive review about gastric microbiota and discuss emerging concepts for the influence of *H. pylori* infection on gastric microbiome and *vice versa*.

Human stomach had been thought to be sterile, and following *H. pylori* discovery, it was thought to be the only bacterium able to colonize the gastric epithelium. Gastric environment is difficult to colonize mainly because of gastric acid barrier. Therefore, the microbial load is much less in the stomach than in the colon ( $10^{10}$ - $10^{12}$  Colony-Forming Units (CFU)/mL) or been small intestine ( $10^2$ - $10^4$  CFU/mL) [2]. Gastric microbiota has previously identified by cultivation of gastric juice or mucosa biopsies. In the healthy stomach, the predominant bacteria belong to the species *Clostridium*, *Lactobacillus* and *Veillonella* based on culture analysis [3]. In the atrophic gastritis with the absence of *H. pylori*, urease producing members of the gastric microbiota, such as *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus capitis*, and *Micrococcus* species can cause false positive results in urea breath tests [4].

Recent advance of molecular biology, computer technology and bioinformatics allow genetic analysis of complex microbiota without cultivation, and the 16S rDNA sequence has opened a field

for extensively revealing novel and uncultivated bacterial species in human stomach [5,6]. In the healthy stomach, the predominant bacteria belong to the phyla of Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (which include *H. pylori*), and genus of *Streptococcus* [7-9]. Utilizing a newer technology, tagged 454 pyrosequencing, analysis of *H. pylori*-negative biopsy samples identified 262 phylotypes representing 13 phyla [10]. The composition of the gastric microbiome in *H. pylori*-negative individuals is highly diverse. Common phylotypes present in *H. pylori*-uninfected subjects include *Streptococcus*, *Prevotella*, and *Gemella* [7]. These findings lend further support to the gastric microbiota being highly diverse, despite significant variability in the microbial composition between individuals [7,8].

In contrast, among *H. pylori*-positive subjects, the microbiota is much more uniform and *H. pylori* represents the most abundant phylotype. *H. pylori* DNA accounts for >90% of all sequence reads in *H. pylori*-positive subjects [7], so it greatly reduces the overall diversity of the gastric microbiota. The most abundant phyla in *H. pylori*-colonized stomachs are Proteobacteria, Firmicutes, and Actinobacteria [10,11]. *H. pylori*-infected adults are likely to have higher abundances of Spirochaetes, Acidobacteria and non-Helicobacter Proteobacteria and relatively lower abundance of Actinobacteria, Bacteroidetes, Firmicutes compared to *H. pylori*-uninfected adults [10]. A study performed by Hu et al. [12] identified the non-*H. pylori* bacterial flora in gastric biopsy specimens taken from *H. pylori* positive patients using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The major species were *Streptococcus*, *Neisseria*, *Rothia*, and *Staphylococcus*, which differed from previous reports of healthy volunteers. Klymiuk et al. [13] recently conducted a prospective, multicenter, clinical trial of 30 gastric biopsy samples including CagA-negative and CagA-positive *H. pylori*. In their study, the genera *Actinomyces*, *Granulicatella*, *Veillonella*, *Fusobacterium*, *Neisseria*, *Helicobacter*, *Streptococcus*, and *Prevotella* are significantly different between the *H. pylori*-positive and negative sample groups. However, there is no significant correlation of *H. pylori* phylogeographic population or carriage of the cag PAI with microbiota composition [13].

Interestingly, Khosravi et al. [14,15] isolated *Streptococcus mitis*

**Table 1:** Human gastric microbiota studies (not including gastric cancer patients).

Authors. year	Sample/Method	Samples	Results
Bik, et al. 2006 [8]	Gastric biopsies 16S rDNA clone library	23 USA adults 12 HP-positive 11 HP-negative (7 contained HP rDNA)	HP seems not affect the gastric microbiome composition.
Andersson, et al. 2008 [7]	Gastric biopsies pyrosequencing of V6 of 16SrRNA	6 healthy Swedish 3 HP-positive 3 HP-negative	Higher diversity in HP-negative patients than HP-positive patients
Li et al. 2009 [9]	Gastric biopsies 16S rRNA sequencing	45 Chinese 22 healthy 23 antral gastritis All HP-negative	Significantly higher abundance of the Firmicutes phylum and the <i>Streptococcus</i> genus was observed in patients with antral gastritis.
Maldonado-Contreras, et al. 2011 [10]	Gastric biopsies PhyloChip	12 adult patients 10 Amerindians 2 immigrants from South Asia and Africa 8 HP-positive	Marked differences were detected in the structure of the gastric bacterial community according to HP status.
Hu et al. 2012 [12]	Gastric biopsies MALDI-TOF MS	103 Chinese patients with dyspeptic symptoms 103 HP-positive	High prevalence of the non-HP bacterial dominated by some species.
Klymiuk, et al 2016 [13]	Gastric biopsies 16S rRNA sequencing	30 Austrian 10HP Cag A-positive 10 HP-Cag A-negative 10 HP-negative	A dramatic decrease of non HP bacterial microbiome in HP infected samples and no significant influence of CagA gene presence.
Parsons, et al. 2017 [19]	Gastric biopsies 16S rRNA sequencing	95 British patients (normal stomach, PPI treated, HP gastritis, HP induced atrophic gastritis and autoimmune atrophic gastritis)	Autoimmune and HP induced atrophic gastritis were associated with different gastric microbial profiles. PPI treated patients showed relatively few alterations in the gastric microbiota compared to healthy subjects.
Pereira, et al. 2018 [30]	Gastric biopsies MALDI-TOF MS	74 HP-positive 21with chronic dyspepsia 53 without	<i>Staphylococcus</i> and <i>Lactobacillus</i> spp. were more commonly identified in patients with chronic dyspepsia.
Schulz, et al. 2018 [33]	Saliva, gastric and duodenal aspirates and biopsies 16S rRNA sequencing	24 German with chronic gastritis 8 HP-positive 16 HP-negative	<i>Helicobacter</i> spp. dominate the mucosa-associated community in the stomach, and to significantly influence duodenal and oral communities.

GC: Gastric Cancer; HP: *Helicobacter pylori*; MALDI-TOF MS: Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry.

(*S. mitis*) and *Lactobacillus fermentum* (*L. fermentum*) from human gastric tissue biopsies, and they have shown that metabolites released by co-culturing *S. mitis* and *H. pylori* induced *H. pylori* to transform into viable but non-culturable coccoidal form *in vitro*. Streptococci seem to survive and develop in an acidic gastric environment as an indigenous microbiota of the gastric mucosa, which may inhibit the colonization by *H. pylori*. In contrast, *L. fermentum* improved *S. mitis* survival *via* secreting diffusible factors [15].

## Hypochlorhydria and Gastric Microbiota

Gastric cancer usually develops *via* gastric atrophy, and the resulting hypochlorhydria potentially leads to alterations in the composition of the gastric microbiota by providing a more favourable environment for colonization. It is generally thought that the hypochlorhydria observed following proton pump inhibitor (PPI) long use does not increase the risk of gastric cancer. Moreover, autoimmune atrophic gastritis is more frequently associated with the development of gastric neuroendocrine Tumor (NET), although it also increases the risk of gastric cancer.

Two recent studies reported by Imhann et al and Jackson et al. have independently identified that long term use of PPI alter the gut microbiota to predispose to enteric infection including *Clostridium difficile* [16,17]. More recent study indicated influence of potassium competitive acid blocker (vonoprazan) on the gut microbiome *H. pylori*-negative healthy individuals. Its stronger effect was reported to be increase in the genus *Streptococcus* as compared with PPI (>20-fold by vonoprazan *vs* approximately seven fold by lansoprazole). In addition, vonoprazan, but not PPI, induced a significant increase in the core members of the oral microbiome, such as the genera

*Actinomyces* and *Rothia*, in the gut microbiome [18].

In a recent British study including 95 patients consisting of five groups (20 normal, 19 PPI treated, 22 *H. pylori* gastritis, 23 *H. pylori* induced atrophic gastritis and 11 autoimmune atrophic gastritis), the microbial profiles in the stomachs of patients with *H. pylori*-induced atrophic gastritis and autoimmune atrophic gastritis were quite different. Interestingly, PPI-treated patients showed more similarities in microbial diversity and abundance to the patients who had autoimmune atrophic gastritis, than to the patients who had *H. pylori*-induced atrophic gastritis [19]. The results agree with other previous reports that PPIs do not significantly influence the gastric microbiota [20,21], however, samples from PPI-treated patients contained significantly more *Streptococcus* at the Operational Taxonomic Unit (OUT) level [22]. Early interactions between *H. pylori* and human tissues may alter the structure of the gastric microbiome, by promoting gastric atrophy.

## Gastric Cancer and Microbiota

Less-acidic conditions lead to new microbiome populations that might promote carcinogenesis, especially development gastric cancer in patients with severe atrophy. Studies examining imbalance of the gastric microbiota in *H. pylori*-associated gastric cancer are more limited. Dicksved, et al. [23] performed 16S rRNA gene sequencing analysis of gastric mucosa of patients with gastric cancer and found that the diversity was equal to the dyspeptic controls. The microbiota of cancer patients was predominantly composed of the genera *Lactobacillus*, *Streptococcus*, *Prevotella*, and *Veillonella*. *S. mitis* and *S. parasanguinis* were the most common species among *S. genera*.

More recently, Eun, et al. [24] investigated the gastric microbiota

**Table 2:** Human gastric microbiota studies (including gastric cancer patients).

Authors. year	Sample/Method	Samples	Results
Dicksved, et al. 2009 [23]	Gastric biopsies T-RFLP 16S rRNA cloning and sequencing	Swedish 10 GC (5 intestinal type, 5 diffuse type; 8 HP-positive) 5 HP-negative dyspeptic controls	Microbiota in cancer patients was dominated by different species of <i>Streptococcus</i> , <i>Lactobacillus</i> , <i>Veillonella</i> , and <i>Prevotella</i> .
Eun, et al. 2014 [24]	Gastric biopsies 454 pyrosequencing of V5 of 16S rRNA	31 Korean patients (11 noncardia GC 10 intestinal metaplasia 10 chronic gastritis) 18 HP-positive	The microbial compositions of gastric mucosa from gastric cancer patients are significantly different to the other groups.
Khosrav, et al. 2014 [14]	Gastric biopsies MALDI-TOF MS 16S rRNA sequencing	215 Malaysian patients (185 FD, 22 PUD 8 GC) 131 HP-positive 84 HP-negative	The presence of HP did not significantly modify the diversity of the gastric microbiota. There may be geographical variations in the diversity of the gastric microbiome.
Yang, et al. 2016 [25]	Gastric biopsies 16S rDNA deep sequencing	40 Colombian 20 High GA risk town 20 low GA risk town 39 HP-positive	The gastric microbiota compositions differed between the two population.
Yu, et al. 2017 [27]	Surgical tissue 16S rRNA sequencing PICRUSt	77 Chinese with operated GC Differentiated 29; Poorly; 48 Metastasis 54	Non-malignant tissue microbiota features were associated with family history of UGI cancer, tumor grade and metastasis.
Li, et al 2017 [29]	Gastric biopsies 16S rDNA V3-V4 sequencing Gastric biopsies 16S rDNA V3-V4 sequencing	33 Korean (Normal 8 Gastritis 9 IM 9; GA 7) 11 HP-positive (before and after eradication)	Eradication resulted in restoration of gastric microenvironment to that of HP-negative subject and an increase in the bacterial diversity index.
Hsieh, et al. 2018 [26]	Gastric biopsies 16S rRNA sequencing	27 Taiwanese 9 gastritis (5 HP-positive) 7 IM (4 HP-positive) 11 GC (3 HP-positive)	<i>Clostridium</i> and <i>Fusobacterium</i> frequently colonize in patients with GC.

GC: Gastric Cancer; HP: *Helicobacter pylori*; MALDI-TOF MS: Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry; FD: Functional Dyspepsia; PUD: Peptic Ulcer Disease; T-RFLP: Terminal Restriction Fragment; PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States.

by pyrosequencing and proposed that there were marked differences in the composition and diversity among patients with gastric cancer, intestinal metaplasia and chronic gastritis, especially in *Helicobacter*-dominant group. The relative abundance of *Helicobacteraceae* family was significantly lower in gastric cancer than chronic gastritis and intestinal metaplasia, while the relative abundance of *Streptococcaceae* family significantly increased. Furthermore, in an Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering of *Helicobacter*-dominant group, the chronic gastritis group and gastric cancer group were clearly separated while the intestinal metaplasia group was distributed between the two groups [24]. Their findings also suggest that the carcinogenic role of *H. pylori* may partly due to its impact on the gastric commensal flora, which become a favorable environment for gastric cancer development.

In a recent study comparing the gastric microbiota between the two Colombian populations; one at high-risk and one at low-risk of developing gastric cancer, significant correlations were found with the town of origin [25]. *Leptotrichia wadei*, which is associated with necrotizing enterocolitis, bacteremia, and a *Veillonella* sp., were significantly more abundant in the high risk population. Interestingly, there was no significant correlation of *H. pylori* phylogeographic population or carriage of the *cagPAI* with microbiota composition [25].

Another study from Taiwan indicated that dominant bacterial species in the *H. pylori*-negative patients were *Burkholderia*, *Enterobacter*, and *Leclercia*. The abundance of those bacteria was similar between the cancer and non-cancer groups, whereas the frequency and abundance of *H. pylori* were significantly lower in the cancer group. *Clostridium colicanis* and *Fusobacterium nucleatum* were significantly more abundant in patients with gastric cancer in *H. pylori*-negative patients [26]. Another recent study investigating non-malignant tissue microbiota using resected stomach due to cancer

indicated that Bacteroidetes was associated with lower tumor grade and *Lactobacillales* was negatively associated with metastasis [27]. Bacteroidetes might be involved in protection against gastric cancer progression, and the most abundant genus was *Prevotella*, which is commonly detected in stomach and oral samples.

Overall, there is emerging evidence of the presence of non-*Helicobacter* bacteria in the human stomach besides *H. pylori*. However, the specific role of individual microorganisms in human gastric carcinogenesis remains unclear, and further studies are needed to investigate the potential role of non-*H. pylori* bacteria in tumor progression.

The results of two previous studies on the bacterial diversity during the gastric cancer progression were conflicting. One study indicated the progressive decline and the other indicated the increase during progression from gastritis to cancer [28,24]. Li, et al [29] first examined the effect of *H. pylori* eradication on gastric microbiota and found that eradication resulted in restoration of gastric microenvironment to that of *H. pylori*-negative subject and an increase in the bacterial diversity index. There was an inverse association between *H. pylori* abundance and bacterial diversity in non-cancer gastric samples including normal, gastritis and intestinal metaplasia, this inverse association was weak in cancer samples. The bacterial diversity seems to be reduced in cancer samples with low *H. pylori* abundance. Eradication may possibly prevent development of gastric cancer by improvement of gastric dysbiosis and restoration of the disturbed gastric homeostasis.

## Chronic Dyspepsia and Microbiota

In a study performed by Hu, et al., the prevalence of non-*H. pylori* bacterial was observed higher in non-ulcer dyspepsia group than in gastric ulcer group indicating the potential roles of non-*H. pylori* bacterial played in the pathogenesis of stomach disorders



[12]. In another recent study investigating gastric microbiota using the same method, *Staphylococcus* spp. and *Lactobacillus* spp. were significantly more commonly identified in patients with chronic dyspepsia; *Streptococcus* spp., *Pseudomonas mosselii*, *Escherichia coli* and *Klebsiella pneumoniae* were more common in non-dyspeptic patients [30]. Some or all of these organisms possibly play a role in the causation of symptoms in *H. pylori* positive patients.

## H. pylori Infection and Intestinal Microbiota

Changes of the microbiota in the duodenum and the proximal small bowel due to *H. pylori* infection have yet to be studied despite the causal relationship between a gastric infection with *H. pylori* and duodenal ulcer disease [31,32]. A recent study by Schulz, et al. [33] investigating the influence of *H. pylori* on duodenal and oral communities indicated the only significant influence of *H. pylori* infection on duodenal communities at the phylum level, which was the increased abundance of Proteobacteria probably due to transfer of *Helicobacter* from the stomach to the duodenal lumen. The influence of *Helicobacter* on the community was more evident in the duodenal samples compared to oral samples, although phylotypes in saliva were different between the *H. pylori*-positive and negative subjects.

In agreement of animal experiments, a study investigating faecal samples obtained from 39 *H. pylori*-infected patients and 19 *H. pylori*-negative volunteers showed that the composition of the microbiota between *H. pylori*-positive versus *H. pylori*-negative control individuals differed with regard to Clostridia and the total number of anaerobes [34]. Another study by Buhling, et al. [35] investigating the intestinal microbiota using faecal samples from 51 *H. pylori*-positive patients found that the microbiota of *H. pylori*-infected patients, characterized by an increase in growth of Lactobacilli, was different from that in *H. pylori* negative controls. However, fecal compartment is predominantly resident in the lumen and influenced by diet, while mucosa-adherent compartment known as mucosa-associated microbiota (MAM) consists of dense cohesive microbial communities that adhere to mucosal surface of GI tract [36]. Thus evaluating MAM in precise and accurate manners is recognized as a clinically important trial to characterize and understand the *H. pylori* and gut microbiome interactions and their roles in the development of health promotion and diseases.

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