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Stomach (P Malfertheiner, Section Editor)

What is the Relevance of Gastric Microbiota Beyond *H. pylori*?

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Abstract

Purpose The role of *Helicobacter pylori* as key factor in gastric inflammation and the development of (pre-)cancerous lesions is undisputable. As an open system, the human upper gastrointestinal tract harbors a complex bacterial community which is highly impacted by the absence or presence of *H. pylori*. The interaction between other bacteria and *H. pylori* might impact on gastric carcinogenesis.
Recent findings Several studies demonstrated differences in the composition of the gastric bacterial community in different stages of gastritis and between samples from tumor and adjacent tissue. In addition, animal studies demonstrated an increased and accelerated development of precancerous lesions in mice colonized with intestinal flora and *H. pylori* compared with mice mono-infected with *H. pylori*.
Conclusion Other bacteria beyond *H. pylori* enter the focus in research on gastric carcinogenesis. However, we are still far from a thorough understanding of the pathophysiology of host-microbiota interaction and its impact on the development of malignant and precancerous changes.

Introduction

The discovery of *Helicobacter pylori* (*H. pylori*) more than 35 years ago signed a turning point in our understanding of gastroduodenal pathologies. While chronic active gastritis is initiated in all subjects infected with *H. pylori* and in its various phenotypic expressions is the basis for the development of several and serious complications, 80% of all infected will not have any progression to complications.

During the last decade, it has become evident that there is a gastric microbiome of high diversity beyond *H. pylori*. Its pathophysiologic role, however, is to be elucidated. Initial evidence for the presence of other bacteria than *H. pylori* in the stomach was made using culturing techniques. These studies were limited by the fact that the majority of intestinal bacteria is anaerobic and therefore difficult to culture. First studies used gastric juice and demonstrated the presence of different phyla as Firmicutes, Proteobacteria, and Bacteroidetes which are known to be present in the entire gastrointestinal tract [1, 2]. In general, the majority of studies published did not analyze the bacterial communities of adjacent ecological niches as oral cavity and stomach in a separated way. So, a discrimination between ingested transient bacteria and the resident bacterial community of the human stomach was not possible. Another methodological issue runs through the published studies: several studies did not address the gastric pH value or histopathological findings causing changes of physiological conditions [3, 4]. Studies published after the launch of acid-suppressive therapy verified distinct changes of gastric microbiota other than *H. pylori* in subjects under acid suppression [5–11].

Advances in the development of culture-independent techniques allow the inexpensive and fast identification and characterization of the bacterial community of different sites and in different materials based on nucleotide sequencing of bacterial 16S rDNA or rRNA [12, 13].

Considering the human intestinal tract as an open system with different adjacent ecological niches, a connection between the oral cavity, the stomach, and the duodenum, and distinct differences between luminal and mucosal bacterial communities were shown [14]. *H. pylori* impacts on the global bacterial community suggesting a connection between other bacteria of the upper gastrointestinal niches and *H. pylori*.

H. pylori—an obligate human pathogen

The human stomach is considered the unique natural reservoir of *H. pylori* in humans. Few publications demonstrated human root canals as potential reservoirs but these findings can also reflect a reflux of bacteria from the stomach [15].

H. pylori contains high amounts of cytoplasmic urease as an important adaptive feature to the hostile environment of the human stomach. Hydrolysis of urea facilitates its survival in the gastric microniche infected [16].

Each subject infected with *H. pylori* develops chronic active gastritis, but only a subset of infected subjects will develop clinical symptoms, and a further subset will develop more severe complications as peptic ulcer disease, gastric MALT-lymphoma, or gastric cancer. Different patterns of gastritis are associated with various clinical presentations of *H. pylori* infection [17, 18]. Moreover, different virulence factors of *H. pylori* are associated with variable risks for the development of complications. Some of the known bacterial factors are associated with gastric cancer and duodenal ulcer disease; e.g., the duodenal ulcer-promoting gene A (DupA) is associated with an increased antral neutrophilic infiltration and reduced risk for gastric atrophy and gastric cancer but is a marker for duodenal involvement. Duodenal ulcer—frequently associated with antral-predominant gastritis—is the most prevalent *H. pylori*-associated complication [19].

H. pylori and its role in gastric carcinogenesis

H. pylori infection is the leading event in gastric carcinogenesis which, however, is a multifactorial process. Based mainly on epidemiological studies showing that the infection with *H. pylori* increases the risk of gastric cancer by factors 2 to 3, the World Health Organization classified *H. pylori* as a class I human carcinogen already in 1994 which was reconciled in 2012 [20, 21]. The evidence has later on been strengthened by in vitro and in vivo studies [22, 23]. With an OR of 21.0, more than 90% of non-cardia gastric cancers are attributable to *H. pylori* infection [24]. For both intestinal-type and diffuse-type gastric cancer, the risk of cancer arising from *H. pylori* infection is similar [25–27].

In intestinal-type gastric cancer, a cascade characterized by Correa describes the progression of chronic gastritis initiated by the infection with *H. pylori* and perpetuated by environmental and host factors to atrophic gastritis, intestinal metaplasia and in some patients to dysplasia, and finally gastric cancer [28]. This cascade was intensively studied in animal models, and it has been addressed in clinical studies whether eradication of *H. pylori* has the potential to stop or even reverse this process and prevent carcinogenesis at any stage of this cascade or whether there is a point of no return.

In Mongolian gerbils, the infection with *H. pylori* induces well-differentiated gastric adenocarcinomas [22, 29]. In a large prospective endoscopic follow-up study on 1526 patients with duodenal or gastric ulcers, gastric hyperplasia, or non-ulcer dyspepsia, of whom 1246 were infected with *H. pylori*, Uemura and colleagues showed a development of gastric cancer just in infected patients. Patients with severe gastric atrophy, corpus-predominant gastritis, or intestinal metaplasia had a significantly increased risk for gastric cancer [30]. A meta-analysis of 19 studies including 2491 patients and 3959 controls revealed an odds ratio of 1.92 (95% CI 1.32–2.78) for gastric cancer in *H. pylori*-infected patients [31]. The complex interplay involved in gastric cancer development further comprises bacterial virulence, host susceptibility genes, and environmental factors [32]. On the bacterial side, the virulence factors CagA and VacA are associated with an increased gastric cancer risk. CagA is translocated into the host cell by type IV secretion system and behaves as a classic oncogene [32]. Polymorphisms and epigenetic alterations in genes encoding factors involved in the inflammatory immune response to the infection and including gene alterations in both the adaptive and the innate immune system including interleukins (IL1β, IL8), transcription factors (CDX2, RUNX3, TLR1), and DNA repair enzymes play a crucial role on the host side [33–36].

H. pylori eradication therapy has the potential to prevent gastric cancer. A meta-analysis of randomized controlled trials revealed that *H. pylori* eradication is a primary chemo-preventive strategy of gastric cancer with an OR of 0.67 (95% CI 0.42–1.07) in favor of eradication therapy [37]. Atrophic gastritis and intestinal metaplasia are possibly reversible in some patients after *H. pylori* eradication, but there might be a point of no return where irreversible genetic and epigenetic changes have already taken place, and the cascade leading to cancer cannot be interrupted anymore as eradication therapy in high-risk situations does not prevent all cases of gastric cancer. However, even in patients already treated for early-stage gastric cancer, *H. pylori* eradication was shown to be still effective in a subset of patients by minimizing the risk for metachronous gastric cancer [38•, 39, 40]. As patients with severe gastric atrophy, corpus-predominant gastritis, or intestinal metaplasia are at high risk for the development of gastric cancer, current guidelines recommend to follow them up regularly with surveillance endoscopies [25, 41].

The human gastric microbiome beyond *H. pylori*—current knowledge

The development of new nucleotide sequencing techniques and bioinformatics tools has augmented our knowledge on gastrointestinal microbiota. In contrast to a longstanding dogma, *H. pylori* is not the only bacterium able to survive in the hostile territorium, but also in the acidic stomach, a diverse microbial community has been detected [42]. Members of the phyla Firmicutes, Proteobacteria, and Bacteroidetes were cultured from gastric juice [43]. Since then, a complex bacterial community has been characterized by several groups overcoming the restrictions of culture-based methods. As the methods applied are highly diverse, comparative analysis of study results is hampered.

Using a small subunit 16S rDNA clone library approach, Bik and co-workers described a diverse community of 128 phylotypes within gastric mucosal samples with the majority of bacteria belonging to Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria phyla. Already in this analysis, a large degree of intersubject variability of the gastric ecosystem was evident [13]. Significant proportions of bacterial microbiota in the stomach originate from the oral cavity being swallowed down [44] and in part just pass the stomach without colonizing or infecting it. Additionally, environmental and dietary changes strongly influence the gastric microbiome [5]. Consistently, studies on the human gastric microbiome indicate a distinct gastric microbial pattern with Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria as the dominating phyla and *Streptococcus* as the most dominant genus [14, 45, 46]

Studies evaluating the impact of *H. pylori* on the composition of gastric microbiota are not fully in line. While the group of Bik did not depict an impact of the presence of *H. pylori* in mucosal samples on the composition of gastric microbiota [13] which was confirmed by a study based on cultures from gastric biopsy samples [47], others characterize *H. pylori* to be the director of the gastric bacterial community.

More advanced studies characterizing not only the gastric microbiota composition but also the continuity of the upper gastrointestinal tract including analyses from saliva and duodenum and applying analyses based on reverse-transcribed 16S rRNA instead of 16S rDNA as template which allows the detection of the metabolically active component of the community affirm a unique microbiota composition in each individual consistent across the different niches. Additionally, these studies characterized *Helicobacter* spp. as dominating the mucosa-associated community in the stomach, and to significantly influence duodenal and oral communities [46, 48••].

A recent study from Mongolia also aimed at a characterization of the gastric mucosal microbiota in *H. pylori* negative compared with *H. pylori*-positive gastritis and to a *H. pylori*-negative non-gastritis control group applying 16S rRNA gene amplicon sequencing. Subjects infected with *H. pylori* had a significantly lower bacterial richness as well as Shannon and Simpson indices as compared with both other groups. The linear discriminant analysis effect size analysis showed the enrichment of Firmicutes, Fusobacteria, Bacteroidetes, and Actinobacteria at phylum level in patients with *H. pylori*-negative gastritis [49].

The application of 16S rRNA sequencing of gastric corpus biopsies from 95 individuals comprising normal stomach, PPI treated, *H. pylori* gastritis, *H. pylori*-induced atrophic gastritis, and autoimmune atrophic gastritis resulted in different gastric microbial profiles in autoimmune and *H. pylori*-induced atrophic gastritis, while PPI-treated patients showed relatively few alterations in the gastric microbiota compared with healthy subjects [50].

The genetic variability of *H. pylori* probably additionally impacts on the composition of gastric microbiota. In a comparative study, *H. pylori*+/CagA+ samples were represented by a decrease in bacterial diversity, a reduced abundance of *Roseburia*, and increased abundances of *Helicobacter* and *Haemophilus* genera. The presence of CagA gene was linked to an increased proportion of Gram-negative bacteria in the stomach [51]. In line with this observation, an Austrian study revealed a trend towards higher abundance of *H. pylori* in subjects infected with CagA-positive strains compared with individuals infected with CagA-negative strains [52].

However, current published evidence does not allow to fully understand the role of the gastric microbiota and its interplay with the host.

Findings suggesting a role of other gastric microbiota in health and disease

Results from animal studies suggest a significant role of other gastric bacteria than *H. pylori* on gastric carcinogenesis. Emerging studies in INS-GAS mice demonstrated a decelarated development of precancerous lesions in *H. pylori*-infected germ-free INS-GAS mice compared with specific pathogen-free INS-GAS mice [53]. Also, the addition of restricted Altered Schaedlers flora, a defined combination of ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus*, and ASF519 *Bacteroides* species, or intestinal flora to *H. pylori*-infected INS-GAS mice led to the development of more severe mucosal changes than in *H. pylori* mono-infected or germ-free INS-GAS mice [54••].

More recent studies aimed at a more diligent characterization of the composition of the gastric bacterial community with respect to histopathological changes of the gastric mucosa. Several studies analyzed human gastric biopsies from histopathologically well-characterized patients, whereas other analyses studied fecal samples from patients with histopathologically staged gastric changes.

Depending on physiological changes in parallel to the development of precancerous lesions, alterations in the microbial composition were shown. Along the Correa cascade, a decreased microbial diversity and a decreased abundance of *H. pylori* was depicted in gastric biopsies from patients with chronic gastritis compared with individuals with gastric cancer [55••]. In gastric carcinoma, microbiota present with an overrepresentation of bacterial genera that include intestinal commensals and have increased nitrosating potential suggesting a role in carcinogenesis due to its genotoxic capability.

A comparison of the microbiota composition of patients with current or past *H. pylori* infection and *H. pylori*-negative individuals taking histopathological changes (gastritis or metaplasia) into account, revealed a significant decrease of Bacteroidetes along different histopathological stages of infection, whereas Firmicutes and Proteobacteria presented with increasing trends throughout the course of infection [56]. A study analyzing not only the gastric microbiome by 16S rRNA sequencing in biopsies from individuals with different gastritis status and *H. pylori* infection status but also performing metagenomics analyses including type IV secretions system (T4SS) genes observed T4SS genes highly in subjects with intestinal metaplasia. They conclude that the abundance of T4SS proteins might promote gastric carcinogenesis in more severe gastritis stages [57].

Studies analyzing the gastric microenvironment with its niche-specific bacterial communities in patients with gastric cancer are rare. A study in patients with esophageal squamous cell carcinoma or gastric adenocarcinoma characterized distinct microbial patterns in nontumor and tumor tissue. In patients with gastric cancer, a decreased abundance of *H. pylori* in tumor tissue was demonstrated [58]. A study from China compared tumor and adjacent tissue samples in 62 gastric cancer patients by 16S rRNA gene sequencing. Differences in composition, structure, interaction networks, and functions of the bacterial community were described. In tumor samples, bacteria from oral origin such as *Peptostreptococcus*, *Streptococcus*, and *Fusobacterium* were predominant while in adjacent tissue, lactic acid producers such as *Lactococcus lactis* and *Lactobacillus brevis* were more abundant. From a functional point of view, a more enhanced bacterial purine metabolism, carbohydrate metabolism, and denitrification functions were detected in the cancer tissue bacterial communities. These findings are consistent with an increased energy metabolism and a higher concentration of nitrogen-containing compounds in the tumor microenvironment. The authors conclude that especially changes in the oral microbiota may play a role in the maintenance of the local microenvironment associated with the development or progression of gastric cancer [59].

Conclusion

Research on the role of other gastric microbiota than *H. pylori* in gastric carcinogenesis is moving on at high pace but is still far from a thorough understanding of pathophysiological pathways.

It has been confirmed in several studies that the presence or absence of *H. pylori* strongly impacts on the composition of the gastric microbiota community. Additionally, differences in the bacterial community at different stages of gastritis and between samples from gastric cancer and adjacent tissue have been characterized.

Well-designed functional analyses within this complex interplay and additionally taking also the adjacent microbial niches into account are still lacking. These will be the challenge for the future.

Compliance with Ethical Standards

Conflict of Interest
Kerstin Schütte, Peter Malfertheiner, and Christian Schulz declare no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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