

# The role of the gastric bacterial microbiome in gastric cancer: *Helicobacter pylori* and beyond

Christian Schulz , Kerstin Schütte, Julia Mayerle and Peter Malfertheiner

**Abstract:** A link between chronic inflammation and carcinogenesis has been depicted in many organ systems. *Helicobacter pylori* is the most prevalent bacterial pathogen, induces chronic gastritis and is associated with more than 90% of cases of gastric cancer (GC). However, the introduction of nucleotide sequencing techniques and the development of biocomputational tools have surpassed traditional culturing techniques and opened a wide field for studying the mucosal and luminal composition of the bacterial gastric microbiota beyond *H. pylori*. In studies applying animal models, a potential role in gastric carcinogenesis for additional bacteria besides *H. pylori* has been demonstrated. At different steps of gastric carcinogenesis, changes in bacterial communities occur. Whether these microbial changes are a driver of malignant disease or a consequence of the histologic progression along the precancerous cascade, is not clear at present. It is hypothesized that atrophy, as a consequence of chronic gastric inflammation, alters the gastric niche for commensals that might further urge the development of *H. pylori*-induced GC.

Here, we review the current state of knowledge on gastric bacteria other than *H. pylori* and on their synergism with *H. pylori* in gastric carcinogenesis.

**Keywords:** gastric bacterial microbiome, *H. pylori*, gastric cancer, microbiota, carcinogenesis

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## Introduction

*Helicobacter pylori* colonizes the human stomach, a unique ecological niche not amenable for colonization by other bacteria. The bacterium is an obligate pathogen, induces chronic gastritis and has been recognized as ‘definite carcinogen’ by the World Health Organization since 1994. Lines of evidence for its role in the development of gastric cancer (GC) were extended and updated in 2012,<sup>1</sup> and *H. pylori* is now considered to be the most prevalent carcinogenic bacterium endangering human health.

The carcinogenic gastric cascade initiated by *H. pylori* is detailed in the Correa sequence of histological changes.<sup>2</sup> Several bacterial virulence factors, host genetic make-up and facilitating ambient, predominantly nutritional, factors

concur in this uneventful process. Robust clinical trials have demonstrated the beneficial effect of GC prevention by *H. pylori* eradication and thus completely proved the carcinogenic role of the bacterium. The role of bacteria other than *H. pylori* has now moved into focus in the study of gastric diseases. The introduction of nucleotide sequencing techniques and the development of biocomputational tools have surpassed traditional culturing techniques and opened a wide field for studying the mucosal and luminal composition of the gastric microbiota. A close connection between gastric microbiota and the bacterial composition in adjacent ecological niches such as the oral cavity and the duodenum has been demonstrated in recent studies.<sup>3</sup> A potential role in gastric carcinogenesis for bacteria other than *H. pylori* has been shown in several experimental

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animal models. However, whether the microbial changes observed in GC are a driver of disease or a consequence of the histologic progression through the precancerous cascade, is not clear at present.

The link between *H. pylori* and GC development has been established using data from epidemiological, basic and translational studies (Table 1). Although GC development is without doubt a result of a complex interplay between host, bacterial and environmental factors, noncardia adenocarcinomas are attributed to *H. pylori* with an odds ratio (OR) of 21.0.<sup>4,5</sup>

In this paper, we review the current state of knowledge on gastric bacteria other than *H. pylori* on the one hand, and on their synergism with *H. pylori* in gastric carcinogenesis on the other.

### Infection-associated cancer: a global burden

Chronic infections are major risk factors triggering carcinogenesis in several organs.<sup>26</sup> About 15% of all diagnosed cancer cases are attributable to infections.<sup>27</sup> The International Agency for Research on Cancer (IARC) has classified a total of 11 infectious agents as group 1 carcinogens, but only *H. pylori* belongs to the domain bacteria.<sup>28</sup> This knowledge and awareness open doors for prevention and therapy of chronic infections to decrease the incidence of infection-associated cancer. Socioeconomic conditions have a major impact on regional differences in risk patterns for infection driven carcinogenesis. While in high and very high developed regions, *H. pylori* is the most relevant infectious carcinogenetic agent, in low and very low developed countries, HHV8, HPV, HBV and HCV play a more dominant role in infection-associated cancers.<sup>29</sup>

Screen-and-treat strategies to prevent GC by *H. pylori* eradication are cost-effective in countries with a high prevalence of GC, but may be extended to countries with an intermediate GC risk.<sup>13,30,31</sup>

### *Helicobacter pylori* in gastric carcinogenesis

The vast majority of noncardia adenocarcinomas is attributed to *H. pylori*. The risk of cardia cancer attributed to *H. pylori* is variable and requires stratification according to the precise topographic

location.<sup>32</sup> The risk of cancer arising from *H. pylori* infection is identical for GC of both the intestinal and diffuse type.<sup>31,33,34</sup>

*H. pylori* was initially classified as a carcinogen based solely on large and well-performed epidemiological studies. The evidence has later been extended and strengthened by *in vitro* and *in vivo* studies.<sup>35,36</sup> Bacterial virulence, host susceptibility genes and environmental factors such as nutritional factors are recognized to be part of a complex interplay involved in GC development.<sup>5</sup>

Different allotypes of bacterial virulence factors such as CagA and VacA are associated with an increased GC risk. CagA is translocated into the host cell by the type IV secretion system and acts as a classic oncogene.<sup>5</sup> On the host side, polymorphisms and epigenetic alterations in genes encoding factors involved in the inflammatory immune response to the infection, including gene alterations in both the adaptive and the innate immune system such as interleukins (IL1 $\beta$ , IL8), transcription factors (CDX2, RUNX3, TLR1) and DNA repair enzymes, play a crucial role.<sup>37–40</sup>

*H. pylori* eradication therapy is effective in preventing GC.<sup>8,9,41–43</sup> In intestinal type GC, carcinogenesis develops stepwise with the transition from chronic atrophic gastritis to intestinal metaplasia, dysplasia to invasive neoplasia (Correa Cascade).<sup>2</sup> 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 if there is a point of no return has been studied intensively (Figure 1). More recently, even in patients being treated for early GC, *H. pylori* eradication was shown to be still effective in a subset of patients by minimizing the risk of metachronous GC.<sup>44,45</sup> Several studies have identified patients with severe atrophic gastritis with and without intestinal metaplasia to be at high risk of developing GC.<sup>46,47</sup> Thus, guidelines recommend that patients with severe gastric atrophy, corpus-predominant gastritis or intestinal metaplasia should be regularly followed up by surveillance endoscopies.<sup>31,48,49</sup>

Later stages of the mucosal damage due to *H. pylori*-induced inflammation might enhance the carcinogenic effect of other risk factors such as other gastric microbiota, salt intake or tobacco smoking.

**Table 1.** Key epidemiological studies supporting the link between *H. pylori* infection and gastric carcinogenesis.

Author	Study design	Result
Hansson <sup>6</sup>	Case control study	The prevalence of <i>H. pylori</i> seropositivity was significantly higher ( $p=0.002$ ) among patients with GC than control patients. The OR was 2.60 (95% CI, 1.35–5.02).
Ekstrom <sup>4</sup>	Population based case-control study	Based on IgG ELISA and CagA seropositivity the OR for noncardia GC among <i>H. pylori</i> -positive subjects is 21 (95% CI, 8.3–53.4)
Uemura <sup>7</sup>	Prospective endoscopic follow-up study	GC develops in persons infected with <i>H. pylori</i> but not in uninfected persons. There is an increased risk for GC in patients with severe gastric atrophy, corpus-predominant gastritis or intestinal metaplasia.
Wong <sup>8</sup>	Prospective, randomized, placebo-controlled, population-based primary prevention study	In <i>H. pylori</i> infected individuals without precancerous lesions, eradication of <i>H. pylori</i> significantly decreases the development of GC.
Fuccio <sup>9</sup>	Meta-analysis	<i>H. pylori</i> eradication is a primary chemo-preventive strategy of GC.
Fukase <sup>10</sup>	Multi-centre, open-label, randomized controlled trial	Eradication of <i>H. pylori</i> after endoscopic resection of early GC has the potential to prevent the development of metachronous gastric carcinoma.
Ma <sup>11</sup>	Prospective randomized controlled trial	GC was diagnosed in 3.0% of subjects who received <i>H. pylori</i> treatment and in 4.6% of those who received placebo (OR=0.61, 95% CI=0.38–0.96, $p=0.032$ ). GC deaths occurred among 1.5% of subjects assigned <i>H. pylori</i> treatment and among 2.1% of those assigned placebo (HR of death=0.67, 95% CI=0.36–1.28).
Li <sup>12</sup>	Prospective randomized controlled trial	Treatment was associated with a statistically significant decrease in GC incidence (OR=0.36; 95% CI=0.17–0.79) and mortality (HR=0.26; 95% CI=0.09–0.79) at ages 55years and older and with a statistically significant decrease in incidence among those with intestinal metaplasia or dysplasia at baseline (odds ratio=0.56; 95% CI=0.34–0.91).
Doorackers <sup>13</sup>	Population based cohort study	Eradication treatment for <i>H. pylori</i> seems to counteract the development of gastric adenocarcinoma and noncardia gastric adenocarcinoma in this Western population.

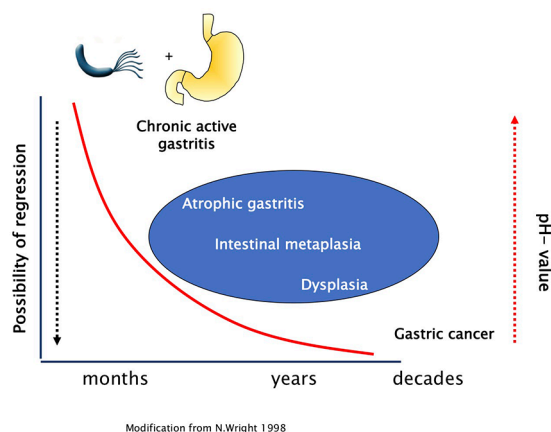
CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GC, gastric cancer; HR, hazard ratio; OR, odds ratio.

### Gastric bacteria other than *Helicobacter pylori* and their role in gastric carcinogenesis

Despite the selective advantage of *H. pylori* to survive in the acidic gastric environment, other bacteria, either as resident community or as transient microbes, interact with the gastric mucosa. Studies based on culturing techniques have demonstrated bacteria other than *H. pylori* in conditions of hypo/achlorhydria that carry an oncogenic potential through their nitrosamine forming functions.<sup>3,33</sup> The availability of high-throughput sequencing permits completely new insights into the gastric microbiota composition (Table 2).

From a historical viewpoint, initial studies on this topic applied culturing methods to stomach

aspirates and documented the presence of members of the phyla Firmicutes, Proteobacteria and Bacteroidetes, which are also dominating in the whole human gastrointestinal tract.<sup>34,35</sup> However, there is a dominance of anaerobic bacteria, which are difficult to culture, in the gastrointestinal tract. Recent comparative studies applying next-generation sequencing (NGS) revealed that the active bacterial community in the gastric mucosa comprises more than 600 bacterial phylotypes and that mainly microbiota communities from the oral cavity are acquired with the use of aspirates from the stomach. The mucosa-associated gastric microbial community is dominated by *Helicobacter* spp. that additionally significantly impact on duodenal and oral communities.<sup>36</sup>



**Figure 1.** Stepwise carcinogenesis in intestinal type gastric cancer.

Several animal studies have demonstrated a potential role in gastric carcinogenesis for additional bacteria besides *H. pylori*. In the absence of intestinal bacteria in germ-free, but *H. pylori*-infected, INS-GAS mice, a reduction in the development of preliminary carcinogenic stages such as atrophic gastritis or intestinal metaplasia was observed. Mice colonised with altered Schaedler flora, including ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus* and ASF519 *Bacteroides* species or intestinal flora and *H. pylori* developed the most severe pathology.<sup>37</sup> These results led to the hypothesis that atrophy as consequence of inflammation alters the gastric niche for commensals that might further urge the development of *H. pylori*-induced GC.

With a clinical approach, gastric biopsies from patients living in an area of Colombia with high risk for the development of GC were compared with matched samples from a distant area with a 25-fold lower risk of GC. Sequencing analysis revealed two significantly more abundant taxa in the high-risk region (*Leptotrichia wadei* and *Veillonella* spp.) whereas *Staphylococcus* spp. were more abundant in the low-risk region. However, a high interindividual variability between all individuals was detected and conclusive interpretation of the findings is not yet possible.<sup>38</sup>

A comparison of gastric microbiota in mucosal biopsies of 54 patients with gastric carcinoma and 81 patients with chronic gastritis by 16S rRNA gene profiling revealed that patients with GC had significantly decreased microbial diversity. Patients

with GC presented with an overrepresentation of non-*Helicobacter* Proteobacteria. The authors conclude that colonization with bacteria other than *H. pylori*, namely gut commensals, contributes to altering the equilibrium between the resident gastric microbiota and the host, and that this dysbiotic microbial community may augment the risk for *H. pylori*-related cancer.<sup>39</sup> Another study also evaluated gastric biopsies from cancer patients compared with chronic active gastritis patients. On the phylum level, no significant differences were detected, which was discussed to be a consequence of reduced acid secretion leading to reduced bacteriocidal capacity of the stomach in patients with GC or advanced stages of gastritis due to the loss of parietal cells.<sup>40</sup> Additionally, a quantitative PCR was used to compare the bacterial load of biopsies in more extensive groups ( $n = 212$  chronic gastritis and  $n = 103$  GC). A significantly increased bacterial load per gram tissue was found in the cancer group, also indicating a loss of hostile conditions in cancerous lesions.

In a 16S rRNA gene analysis of gastric mucosal samples from 81 cases including superficial gastritis (SG), atrophic gastritis, intestinal metaplasia and GC from China, which was validated in a Mongolian cohort, significant mucosa microbial dysbiosis in subjects with intestinal metaplasia and GC was observed with significant enrichment of 21 and depletion of 10 bacterial taxa in GC compared with SG [ $q < 0.05$  after adjusting  $p$ -values for multiple comparisons by the false discovery rate (FDR) method]. Important roles for *P. stomatis*, *D. pneumosintes*, *S. exigua*, *P. micra* and *S. anginosus* in gastric cancer progression were suggested.<sup>41</sup> Another study evaluated 33 individuals including subjects with *H. pylori*-associated chronic gastritis, gastric intestinal metaplasia, gastric adenocarcinoma and *H. pylori*-negative controls. Microbiota in the stomach were analyzed by Illumina MiSeq platform targeting the 16S rDNA from gastric biopsies. A strong negative correlation between *H. pylori* relative abundance and bacterial diversity was observed. In samples from patients with GC, this inverse association was weak. These samples tended to have lower bacterial diversity compared with other samples with similar *H. pylori* levels. After *H. pylori* eradication therapy, bacterial diversity increased, and the relative abundance of other bacteria to levels similar to individuals without *H. pylori* was restored.<sup>50</sup>

**Table 2.** Key findings from pivotal studies on other bacteria than *H. pylori* in gastric carcinogenesis.

Author	Study design	Methods	Key findings
Sharma <sup>14</sup>	Prospective interventional study	Ten healthy volunteers before, during, and after treatment with omeprazole 30 mg daily for 2 weeks. Culture of gastric juice.	Significant increases in the bacterial count and the nitrite and N-nitrosamine concentrations in the gastric juice after PPI ( $p < 0.001$ ).
Mowat <sup>15</sup>	Prospective interventional study	Gastric juice pH; nitrite, and colonization by other bacteria were examined before and during omeprazole treatment in subjects with and without HP infection. Culture.	During omeprazole, <i>H. pylori</i> -positive subjects had a higher intragastric pH (7.8 versus 3.0; $p < 0.00001$ ) and greater colonization with non-HP species ( $5 \times 10^7$ versus $5 \times 10^5$ CFU/mL; $p < 0.05$ ) including nitrosating species.
Sanduleanu <sup>16</sup>	Cohort-study	145 patients on continuous acid inhibition (PPI or H2RA) and 75 dyspeptic patients without acid inhibition. Fasting gastric juice was obtained for pH measurement and bacteriological culture. Gastric biopsy specimens were examined for detection of HP and of non-HP bacteria. Culture and histology.	Both luminal and mucosal growth of non-HP bacteria were significantly greater in HP-positive than -negative patients taking PPI ( $p < 0.05$ for both). Luminal growth of non-HP flora increased with the intragastric pH level.
Schulz <sup>17</sup>	Cohort study	Saliva, gastric and duodenal aspirates as well as gastric and duodenal biopsies from 24 patients (m:9, f:15, mean age $52.2 \pm$ SD 14.5 years). RNA was extracted and the V1–V2 region of the retrotranscribed bacterial 16S rRNA amplified and sequenced on the Illumina MiSeq platform.	687 bacterial phylotypes that belonged to 95 genera and 11 phyla were observed. Each individual comprised a unique microbiota composition that was consistent across the different niches. The stomach fluid enriched for specific microbiota components.
Lertpiriyapong <sup>18</sup>	Mouse model	Gastric colonization with ASF and HP were correlated with pathology, immune response and mRNA expression for proinflammatory and cancer-related genes in germ-free (GF), HP monoassociated (mHp), restricted ASF (rASF; 3 species), and specific pathogen-free (complex IF), hypergastrinemic INS-GAS mice 7 months postinfection. Quantitative PCR (qPCR) for ASF and HP levels	Colonisation with HP and a restricted microbiota consisting of only three species of commensal bacteria promoted gastric cancer in gnotobiotic male INS-GAS mice to a similar extent as mice colonized with complex microbiota. Gastric colonization with complex microbiota lowered gastric HP colonization in both male and female INS-GAS mice.
Jo <sup>19</sup>	Cohort study	Gastric microbiota of 63 antral mucosal and 18 corpus mucosal samples bar-coded 454 pyrosequencing of the 16S rRNA gene (targeting the V1–V3 regions, on GS Junior Sequencing System) with focus on bacteria other than Hp, especially nitrosating or nitrate-reducing bacteria (NB).	The number of NB other than Hp (non-Hp-NB) was two times higher in the cancer groups than in the control groups, but it did not reach statistical significance.
Ferreira <sup>20</sup>	Retrospective cohort study	54 patients with gastric carcinoma and 81 patients with chronic gastritis 16S rRNA gene profiling, using next-generation sequencing (targeting the V5–V6 hypervariable regions, sequenced in an Ion PGM Torrent platform). Associations between the most relevant taxa and clinical diagnosis validated by real-time quantitative PCR	The gastric carcinoma microbiota was characterized by reduced microbial diversity, by decreased abundance of Hp and by the enrichment of other bacterial genera, mostly represented by intestinal commensals. The combination of these taxa into a microbial dysbiosis index revealed that dysbiosis has excellent capacity to discriminate between gastritis and gastric carcinoma. The functional composition of the total gastric carcinoma microbiota had increased nitrate reductase functions.

(Continued)



Table 2. (Continued)

Author	Study design	Methods	Key findings
Wang <sup>21</sup>	Cohort study	315 patients (212 patients with chronic gastritis, 103 patients with gastric cancer) bacterial load of gastric mucosa was determined using quantitative PCR amplicons of the 16S rRNA gene (variable V1–V3 region) from 12 patients were pyrosequenced on a 454 GS-FLX system.	The amount of bacteria in gastric mucosa was estimated to be $6.9 \times 10$ per gram tissue on average. It was higher in HP-infected patients ( $7.80 \pm 0.71$ ) compared with those uninfected ( $7.59 \pm 0.57$ , $p=0.005$ ). An increased bacterial load up to $7.85 \pm 0.70$ was detected in gastric cancer compared with chronic gastritis ( $p=0.001$ ).
Yang <sup>22</sup>	Matched case control study	20 individuals from 2 towns (one with high, the other with low incidence of gastric cancer), matched for age and sex a fragment of the conserved 16S rDNA gene was amplified with a set of broad range primers recognizing highly conserved sequence motifs, the amplicons were sequenced using Roche 454 FLX + technology	The gastric microbiota composition was highly variable between individuals, but showed a significant correlation with the town of origin. Multiple OTUs were detected exclusively in either Tumaco or Túquerres. Two operational taxonomic units (OTUs), <i>Leptotrichia wadei</i> and a <i>Veillonella</i> sp., were significantly more abundant in Túquerres, and 16 OTUs, including a <i>Staphylococcus</i> sp. were significantly more abundant in Tumaco
Coker <sup>23</sup>	Cohort study	81 cases including superficial gastritis (SG), atrophic gastritis (AG), intestinal metaplasia (IM) and GC from Xi'an, China 16S rRNA gene analysis (targeting V4 hypervariable regions) of gastric mucosal samples sequencing on Illumina MiSeq platform	significant mucosa microbial dysbiosis in IM and GC subjects, with significant enrichment of 21 and depletion of 10 bacterial taxa in GC compared with SG ( $q < 0.05$ )
Liu <sup>24</sup>	Retrospective cohort study	276 GC patients without preoperative chemotherapy, and 230 normal, 247 peritumoral and 229 tumoral tissues obtained for gastric microbiota analysis targeting V3–V4 regions of 16S rRNA gene by MiSeq sequencing	GC-specific stomach microhabitats, not GC stages or types, determine the composition and diversity of the gastric microbiota. Bacterial richness was decreased in peritumoral and tumoral microhabitats, and the correlation network of abundant gastric bacteria was simplified in tumoral microhabitat. HP, <i>Prevotella copri</i> and <i>Bacteroides uniformis</i> were significantly decreased, whereas <i>Prevotella melaninogenica</i> , <i>Streptococcus anginosus</i> and <i>Propionibacterium acnes</i> were increased in tumoral microhabitat. Higher HP colonisation influenced the overall structure of the gastric microbiota in normal and peritumoral microhabitats.
Ling <sup>25</sup>	Retrospective cohort study	64 GC patients without preoperative chemotherapy, and 60 normal, 61 peritumoral and 59 tumoral tissues were obtained for gastric mucosal microbiota analysis and immunohistochemistry analysis. Illumina sequencing-with PCR primers 319F/806R, targeting the V3–V4 regions of 16S rRNA gene	diversity, composition and function of gastric mucosal microbiota changed more significantly in tumoral tissues than those in normal and peritumoral ones. Several nonabundant genera such as <i>Stenotrophomonas</i> and <i>Selenomonas</i> were positively correlated with BDCA2 <sup>+</sup> pDCs and Foxp3 <sup>+</sup> Tregs, respectively, while <i>Comamonas</i> and <i>Gaiella</i> were negatively correlated with BDCA2 <sup>+</sup> pDCs and Foxp3 <sup>+</sup> Tregs

AG, atrophic gastritis; ASF, Altered Schaedler's flora; CFU, colony forming units; GC, gastric cancer; HP, *Helicobacter pylori*; IM, intestinal metaplasia; PPI, proton pump inhibitor; SG, superficial gastritis.

A comparison of gastric microbiota composition between normal tissue, peritumoral and tumoral tissues in a cohort of 276 patients with GC with an approach targeting the 16S rRNA gene by

MiSeq sequencing, bacterial richness was lowered in peritumoral and tumoral microhabitats. Additionally, the tumoral microhabitat presented with a simplified correlation network of abundant

gastric bacteria. *H. pylori* (HP), *Prevotella copri* and *Bacteroides uniformis* were less prevalent, whereas *Prevotella melaninogenica*, *Streptococcus anginosus* and *Propionibacterium acnes* were more abundant in tumoral microhabitat.<sup>43</sup> The same group focused on the tumor-immune environment in relation to gastric microbiota in GC patients in a second analysis and depicted a correlation of regulatory T cells and plasmacytoid dendritic cells within the tumor microenvironment with gastric microbiota dysbiosis.<sup>44</sup>

Nitrate-reducing bacteria are considered to aggravate gastric carcinogenesis in addition to *H. pylori* infection. Therefore, Jo and colleagues analyzed the bacterial composition in gastric biopsies of different sites, and divided the cohort into four subgroups (cancer  $\pm$ , *H. pylori*  $\pm$ ). Using pyrosequencing of the 16S rRNA gene with special focus on nitrate-reducing bacteria, no significant differences were detected.<sup>45</sup> Later, one of the studies mentioned above revealed increased nitrate reductase functions promoting the reduction of nitrate to nitrite in addition to increased nitrite reductase functions promoting the reduction of nitrite to nitric oxide in gastric carcinoma microbiota in comparison to microbiota in chronic gastritis.<sup>39</sup>

Other authors bring up the hypothesis of a potential role of biofilm formation in the development of GC, but convincing data to support this assumption is still lacking.<sup>51,52</sup>

However, the analysis of gastric microbiota with sequencing approaches is impeded by the high content of human DNA in mucosal samples, which confounds microbial identification. This can in part be overcome if whole genome sequencing approaches are applied combined with intensive human DNA filtering methods. A study using whole genome sequencing, however, revealed highly consistent results in terms of microbiome profiling of endoscopic biopsy samples with qPCR quantification of *H. pylori* and universal 16S bacterial quantification.<sup>53</sup> Furthermore, a comparison of published sequencing data on the bacterial gastric microbiome is further hampered by methodological differences in published studies with respect to the target of analysis (bacterial RNA or DNA), amplification and extraction methods.<sup>54</sup>

Summarizing current knowledge, the hypothesis is raised that the microenvironment modification,

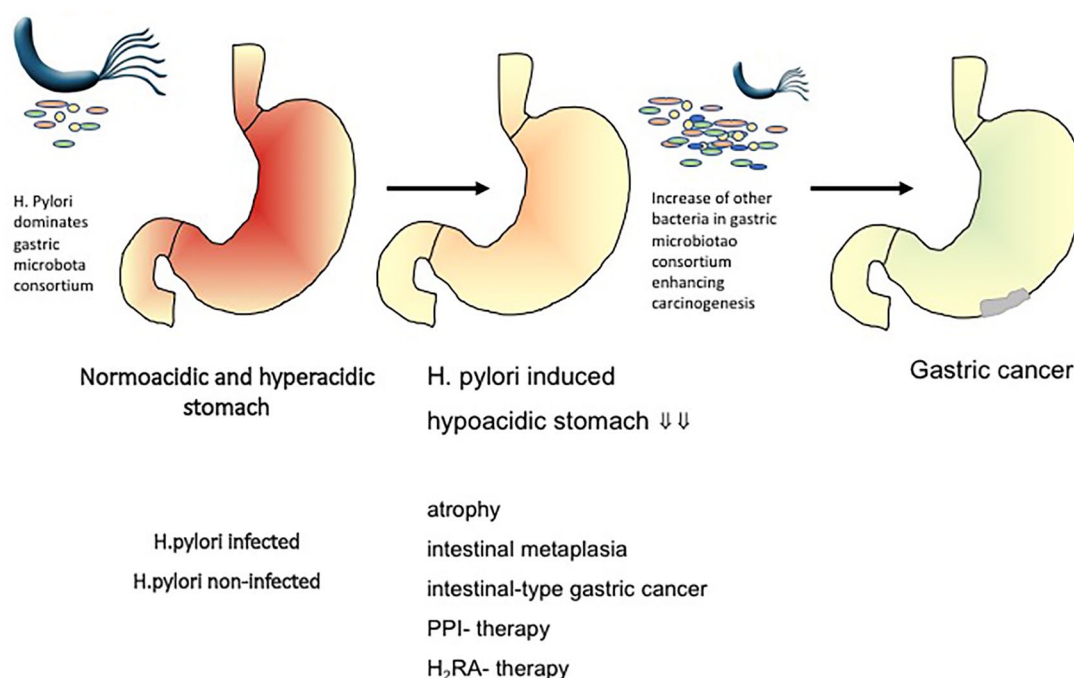
as consequence of chronic atrophic gastritis with atrophy and reduced acidity, results in *H. pylori* substitution by a cancer-prone microbiota. As a consequence, it is discussed that *H. pylori* infection is exclusively linked to a premalignant phase of chronic gastritis, but that the tremendous shifts in gastric microbiota composition in later stages play a more relevant role in carcinogenesis itself. This might have implications for clinical management (Figure 2).<sup>55</sup> The complex interplay between the bacterial gastric microbiota, other players of the microbiome and the host and its immune system in gastric carcinogenesis, however, is far from being fully understood.

### Role of eradication therapy in gastric cancer prevention and interplay with gastric microbiota

Eradication of *H. pylori* is associated with a reduced risk of GC in Asian populations, but also in Western populations with lower incidence of the disease.<sup>11,56</sup> Whether this is a consequence of the eradication of the carcinogen or of the alterations of the whole gastric microbiota following eradication in the short and long term, cannot be answered at this time. A study in 10 asymptomatic young adults compared the structure of the gastric microbiota before and after bismuth quadruple therapy. It demonstrated an increased alpha diversity after eradication over time with an increase in *Lactobacillus* and *Bifidobacterium*, which are assumed to act beneficially.<sup>57</sup> This is in line with findings from a study mentioned before revealing that *H. pylori* infection results in alterations of gastric microbiota and reduction in bacterial diversity, which can be restored by eradication treatment.<sup>50</sup>

### Conclusion

To date, not a single published trial provides convincing evidence for a strong involvement of bacteria other than *H. pylori* in human gastric carcinogenesis, albeit several studies have revealed differences in gastric microbiota composition between healthy individuals, patients with chronic gastritis and GC patients. The functional role of the changes in bacterial microbiota composition observed in advanced gastritis still needs to be elucidated. Additionally, a proportion of GC is associated with EBV infection.<sup>58</sup> Incorporation of the virome and mycome into the complex picture of gastric carcinogenesis is a further challenge.



**Figure 2.** Hypothesis on the impact of other gastric microbiota on gastric cancer development (adopted from Schulz and colleagues).<sup>3</sup>

Future studies not only need to differentiate between active resident and transient bacteria, to prove their mucosal adherence or intracellular localization but to also take the whole complexity of microbiota into account. Additionally, the host response to the alterations observed needs to be characterized.


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### Conflict of interest statement

The authors declare that there is no conflict of interest.

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