Microb Health Dis 2021; 3: e592

European Helicobacter & Microbiota Study Group

HELICOBACTER PYLORI AND THE GUT MICROBIOTA

B. Linz, S. Backert

Division of Microbiology, Department of Biology, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany

Corresponding Author: Steffen Backert, PhD; email: Steffen.Backert@fau.de

Abstract: The gut microbiota inherits fundamental functions in the preservation of human health properties as they are crucial for nutrient acquisition and digestive properties, energy metabolism, and training of host immunity functions. Colonization of the human stomach by the gastric pathogen *Helicobacter pylori* and corresponding antibiotic treatment regimens have a great impact on the number and composition of the gut microbiota. Recently, treatment with probiotic bacteria come into focus as nutritional supplements for *H. pylori* suppression and to boost the success rate of antibiotic treatment against the pathogen, to mitigate the gut dysbiosis and to accelerate the restoration of the gut microbiota. The present article reviews the most recent literature between April 2020 and March 2021 about the impact of *H. pylori* infection and its antimicrobial treatment on the gut microbiota.

Keywords: *Helicobacter pylori*, Antibiotics, Antimicrobial therapy, Microbiota, Probiotics.

INTRODUCTION

Myriads of different microbes, called the gut microbiome, inhabit the human gastrointestinal tract[1]. The most prominent microbes in the healthy gut comprise the phyla Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria[2,3]. Humans are commonly colonized by the microbiota during childhood, with the delivery mode, dietary styles, environmental conditions, host genetics, and age influencing the detailed composition of the microbiota[4]. For a long time, the stomach has been assumed to represent a sterile habitat due to the acidic pH. This view changed radically after the discovery of *Helicobacter pylori* by Barry Marshall and Robin Warren[5], and of other gastric microbiota that in part transiently colonize the human stomach as reported several years later[6]. Today, we know that *H. pylori* colonizes the stomach of approximately half of the world population, with different incidence rates between countries and regions[7]. Once colonized by *H. pylori*, humans can develop a chronic infection that does not eliminate itself, and without active intervention, infected people usually carry the pathogen throughout their life. Most people remain clinically asymptomatic after the initial inflammation step following the colonization event. However, *H. pylori* infection is associated with the development of chronic gastritis, which in turn promotes the progression to peptic ulcers, dyspepsia, gastric cancer and MALT (mucosa-associated lymphoid tissue) lymphoma in a subset of patients[8,9]. Therefore, early effective treatments are recommended to prevent the manifestation of gastric diseases[10].

In recent years, the focus on studying gut microbiota increased due to their health importance, including host metabolism and immunity[1]. Gut bacteria aid greatly in digestion, produce essential biomolecules, such as vitamins that cannot be synthesised by the host himself,

and exhibit immunomodulatory activity[11]. Altering the composition of gut microbiota, for example by treatment with antibiotics, can cause severe inflammation. Reduction or loss of microbiota can even lead to the development of metabolic syndromes. By removing the gut microbiota, antibiotic treatment can lower the resistance of the host against colonization by pathogenic bacteria through opening up ecological space for (opportunistic) pathogens, which can pave the way for serious infections[12]. On the other hand, infection with a variety of pathogens can disturb the microbial community and lead to long-term changes in the composition of the microbiota, particularly infection with pathogens that cause chronic infections such as *H. pylori*. In the present overview article, we review the literature between April 2020 and March 2021 on the complex relation of *H. pylori* and gut microbiota, including the effects of *H. pylori* infection on gut microbiota composition and diversity, the consequences of *H. pylori* eradication therapy on the microbiota, and the possible role of probiotics in anti-*H. pylori* treatment regimens.

IMPACT OF *H. PYLORI* PRESENCE IN THE STOMACH ON THE MICROBIOTA IN THE GUT

Most identified bacteria in the microbiome of stomach mucosa from *H. pylori*-negative individuals in Sweden, a country with low *H. pylori* incidence[7], were assigned to the predominant five phyla *Firmicutes* (42% of all sequencing reads), *Bacteroidetes* (24%), *Proteobacteria* (17%), *Actinobacteria* (7%), and *Fusobacteria* with 6% of the reads[3]. The samples were dominated by bacteria from the genera *Streptococcus* (phylum *Firmicutes*, 24% of the total reads), *Prevotella* (*Bacteroidetes*, 23%), *Veillonella* (*Firmicutes*, 6%), *Fusobacterium* (*Fusobacteria*, 5%), *Haemophilus* (*Proteobacteria*, 4%), *Neisseria* (*Proteobacteria*, 4%), and *Gemella* (*Firmicutes*, 4%). These data are in agreement with previous analyses that were summarized in 2020 by Ozbey et al[6] and by Rajilic-Stojanovic et al[2]. These recent reviews showed that *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* are the predominant taxa in the stomach mucosa, but their proportion differed among various studies and geographical regions, with any of the first three taxa being the predominant group, comprising up to 54% of the total reads. While *Prevotella*, *Streptococcus*, *Veillonella*, *Neisseria*, *Fusobacterium* and *Haemophilus* were the most frequently reported genera, data on the diversity of gastric microbiota were not uniform, indicating regional differences in the abundance of the microbial groups[2]. An extreme predominance of *Proteobacteria* (83.1% of the total reads, mostly from genera *Pseudomonas*, *Serratia*, and *Acinetobacter*), was recently detected in the gastric mucosa of *H. pylori*-negative children from China[13]. Given that the probands in almost all other studies were adults, this brings into question to what extent age influences the stomach microbiome in addition to environment, culture, and diet.

Based on a study[14] about gastric microbiota and *H. pylori* in eight ethnicities in Indonesia from Sumatra, Java, Bali, Sulawesi, Kalimantan, New Guinea, and Timor, the mucosal microbiome in the stomach can also be expected to differ on a local scale. While the α-diversities of samples from *H. pylori*-negative individuals were comparable among the ethnic groups, the β-diversity was significantly associated with the ethnicities, suggesting the presence of unique or predominant taxa. A linear discriminant analysis effect size (LEfSe) revealed specific association of taxa with ethnicities, for example *Streptococcus* and *Micrococcus luteus* with Timorese, *Sphingomonas yabuuchiae* with Papuans, *Bulledia* and *Atopobium* with Javanese. Overall, the relative abundances of *Bulledia*, *Prevotella nanciensis*, and *Prevotella intermedia* were significantly different among the various ethnic groups[14].

Interestingly, the microbial diversity was also affected by the anatomical location in the stomach. A study by Deng et al[15] on the mucosa-associated microbiota, showed vast differences in the microbial composition at the antrum, corpus, and cardia in *H. pylori*-negative patients with chronic gastritis. Among the three anatomical sites the relative abundance of *Proteobacteria* and *Firmicutes* were significantly different. While mucosal samples from the corpus were dominated by *Proteobacteria* (80%, mostly genus *Acinetobacter*) and *Bacteroidetes* (7%), samples from the cardia carried *Proteobacteria* (49%, mostly *Klebsiella* and *Escherichia*), *Firmicutes* (29%, predominantly *Streptococcus*), and *Actinobacteria* (6%), while antrum mucosal samples showed a much more even distribution of *Proteobacteria* (38%, mostly *Citrobacter*), *Firmicutes* (37%, mostly *Streptococcus*), and *Actinobacteria* (15%, mostly *Rothia*)[15].

In agreement with previous reports, several recent studies[2,3,6,14] showed that *H. pylori* infection significantly affects the gastric microbial diversity and composition. In general, infection with *H. pylori* lowered the overall α-diversity, affecting both richness and evenness of the operational taxonomic units (OTUs) in the stomach compared to samples from *H. pylori*-negative and asymptomatic control patients. Interestingly, not only *H. pylori* but also non-helicobacter gastritis affected the microbial diversity, although less pronounced. A study by Ndegwa et al[3] on the gastric microbiota in patients with and without gastrointestinal symptoms showed that the α-diversity was highest in normal healthy stomachs, followed by stomachs of patients with antral non-helicobacter gastritis, whereas the α-diversity in those with atrophic gastritis and chronic *H. pylori* gastritis was significantly lower. Disease progression appeared to decrease the mucosal microbial diversity further, with the α-diversity being highest in patients with a normal healthy stomach mucosa, lower in patients with gastritis, and lowest in patients with intestinal metaplasia, precancerous lesions, and gastric cancer (GC)[2,14]. There was an increasing abundance of pathogenic bacteria along the progression from normal mucosa to the potential early pre-cancerous state, indicating that non-*H. pylori* microbiota appear to contribute to and exacerbate dysbiosis.

Which taxa of the gastric microbiota are associated with the development of gastric cancer? Similar to the diverse composition of the gastric microbiota across the studies, the individual analyses revealed different results. A case control study in Mongolian patients[16] that showed decreased microbial diversity in gastric mucosa from patients with intestinal metaplasia and with GC compared to normal gastric mucosa, revealed an association of the genera *Lactobacillus*, *Paeniglutamicibacter*, *Glutamicibacter*, *Helicobacter*, *Enterococcus*, and *Carnobacterium* with GC. Deng et al[15] analyzed samples sequentially taken from patients in whom gastritis had progressed into gastric cancer. The authors observed a considerable but not significant increase (from 38% to 60%) in *Proteobacteria* (mostly of the order *Pseudomonadales*) in samples from antrum gastric cancer compared to samples from antrum gastritis, which was accompanied by a significant decrease in *Actinobacteria* from 15% to less than 1% of the total sequencing reads. In patients with *H. pylori*-negative antrum GC *Pseudomonadales* and *Erysipelotrichales* were enriched, while samples from patients with *H. pylori*-positive antrum GC revealed enrichment of *Neisseriales*[15]. A further study[17] observed enrichment of *Capnocytophaga*, *Bacillus*, and *Prevotella* after disease progression to dysplasia and GC, while the relative abundance of *Helicobacter* declined.

Gastric adenocarcinoma associated with *H. pylori* infection not only result in dysbiosis of the gastric microbiota, but also affect the intestinal microbiota. A Finnish study[18] analyzed differences in stool microbiota among patients with different histological subtypes of gastric adenocarcinoma, gastric gastrointestinal stromal tumors (GIST), and healthy controls. 16S rRNA sequencing of fecal samples showed the highest microbial diversity in healthy controls, followed by intestinal type adenocarcinoma patients and GIST patients, and the α-diversity was lowest in patients with diffuse adenocarcinoma. All types of gastric tumors were associated with higher abundance of *Enterobacteriaceae* and lower abundance of *Lactobacillaceae* and *Oscillibacter*, which led the authors to suggest that the observed association of higher *Enterobacteriaceae* abundance of with gastric malignancies could potentially be developed into a marker of GC[18]. However, this conclusion was not supported by another study[19] that analyzed bacteria in dyspeptic and GC patients from Denmark and Lithuania. Instead, the proportion of genera *Streptococcus*, *Lactobacillus*, *Gemella*, and *Enterococcus* (all *Firmicutes*) was increased in GC patients compared to dyspeptic patients, while the relative proportion of the genera *Actinomyces*, *Staphylococcus*, and *Corynebacterium* was decreased. A further paper[20] reported a statistically significant association of *Fusobacterium nucleatum* with the survival changes of GC patients with significantly worse overall survival changes of *F. nucleatum*-positive patients compared to *F. nucleatum*-negative patients. In contrast, there was no association of the *F. nucleatum* status with chronic gastritis or intestinal metaplasia.

In contrast to gastric samples, in which *H. pylori* infection decreased the microbial diversity, the α-diversity in the duodenum was increased[13,21-23]. A 2020 research paper from Ecuador[21] on the duodenal microbiome in dyspeptic patients showed significantly higher α-diversity in patients with *H. pylori* infection compared to uninfected patients, results that are in agreement with recent data from Korea[23] and Japan[22]. The β-diversity showed significant differences in the microbiota of *H. pylori*-positive and negative groups, with a higher relative abundance

of *Ralstonia* bacteria in mucosa samples from non-infected subjects, and increased co-occurrence of genera *Haemophilus*, *Neisseria*, *Prevotella* (all *Proteobacteria*) and *Streptococcus* (*Firmicutes*) in samples with *H. pylori*[21]. There was a general predominance of genera, such as *Ralstonia*, *Pseudomonas*, *Streptococcus*, *Haemophilus*, *Neisseria* and *Veillonella*[21], the latter four of which were also among the ten most abundant genera in the duodenal microbiome in a Japanese study by Kashiwagi et al[22].

A Chinese study[13] showed a decreased α-diversity in the stomach mucosa of school children upon infection with *H. pylori*, with on average 30% of the sequencing reads originating from *H. pylori*. In the duodenum, however, in which *H. pylori* made up on average less than 3% of the total microbial abundance, the α-diversity between *H. pylori*-positive and *H. pylori*-negative samples was similar, with largely similar proportions of the major phyla of duodenal microbiota. As a result, the β-diversity of gastric microbiota with and without *H. pylori* infection was significantly different, while a Principal coordinate analysis plot of the duodenal microbiota showed no effect of *H. pylori*[13]. Again, as several other studies showed an increased α-diversity in the duodenum of patients carrying *H. pylori*, this analysis on samples from school-aged children in China raises the question on the importance of age on the dynamics of the microbiome composition.

It is puzzling that *H. pylori* infection decreased the microbial diversity in the stomach but increased the diversity in the duodenum, tempting us to speculate why. In the stomach, *H. pylori* bacteria are highly abundant during infection, which lowers the relative proportion of other bacteria to the point that numerous minor taxa are no longer detected, and thus results in a decreased α-diversity. In contrast, most studies on the effect of *H. pylori* infection on the duodenal microbial diversity reported a relatively low abundance of *H. pylori* among the microbial taxa. Additionally, we hypothesize that manipulation of the pH in the duodenal mucosa by *H. pylori* urease might promote colonization by intestinal bacteria that are normally not found in this relatively acid environment. Future studies are required to address this intriguing question.

Bacteria normally associated with the oral cavity were occasionally found in the gastric mucosa. To assess whether oral bacteria could differentially colonize the gastric mucosa in gastric cancer patients *vs.* patients with superficial gastritis, a study from China analysed bacteria present in paired gastric mucosa and tongue coating samples[24]. The composition of the microbiomes of the gastric mucosa and tongue coating differed significantly; while *Neisseria*, *Prevotella* and *Veillonella* were the most abundant taxa in the tongue coating samples, *Sphingomonas*, *Helicobacter*, and *Caulobacteraceae* genera were dominant in the gastric mucosa. Tongue coating samples and gastric mucosa samples were more similar to one another in gastritis patients relative to GC patients. Many genera were present in both oral and gastric samples, but at different abundance. Of the 15 most abundant tongue coating bacterial genera, six were enriched and nine were depleted in the gastric mucosa. Relative to gastritis patients, three genera of those 15 were enriched in the gastric mucosa of GC patients whereas 12 occurred at lower abundance, showing that co-occurrence of bacteria between the tongue coating and gastric mucosa samples differed between the groups[24].

ANTI-H. PYLORI TREATMENT AFFECTS THE GUT MICROBIOTA

Previously, the standard treatment procedure for *H. pylori* was the two-week triple therapy, prescribed for 2 weeks, which involves two antibiotics (clarithromycin, amoxicillin or metronidazole) combined with a proton pump inhibitor (PPI). However, at present this protocol is only recommended in those regions where clarithromycin resistance is low, but unfortunately the prevalence of resistant isolates is rising. Therefore, quadruple therapies are the treatments of choice in most countries with the goal to increase the treatment success rate. Thus, *H. pylori* eradication regimens usually include two antibiotics (most often metronidazole and tetracycline), bismuth and a PPI (bismuth-quadruple therapy), or even three antibiotics (amoxicillin, clarithromycin and metronidazole) and a PPI (non-bismuth quadruple therapy)[25]. Yet, the success rate of antibiotic therapy is almost never 100%, failing in some patients for a number of reasons, that foremost include increasing *H. pylori* resistance to the various antibiotics, but also high bacterial loads, high gastric acidity, and compliance during

treatment. A recent study[26] on refractory *H. pylori* after two and three eradication failures revealed an extremely high antimicrobial resistance of the persistent strains, which showed a resistance rate of 65.9% for metronidazole, 85.4% for levofloxacin, and 92.7% for clarithromycin. In addition to double resistance for both levofloxacin and clarithromycin in 73.2% of the analysed isolates, 34.1% were resistant to amoxicillin, and 29.3% to rifabutin. This study highlighted the challenges of multidrug resistant *H. pylori* in clinical practise and suggested that a tailored eradication strategy based on the results of antibiotics susceptibility analysis may be the optimum treatment for refractory *H. pylori* infection[26].

The effects of bismuth quadruple therapy on the gut microbiome of children were analysed in another recent study[27] by 16S rRNA sequence analysis of faecal samples collected at two, six, and 52 weeks post antibiotics therapy. The microbial diversity at baseline at the beginning of the study showed no significant differences between the gastritis group, the duodenal ulcer (DU) group and the control group. At week two, compared to baseline, α-diversity was reduced significantly in the gastritis patients, and was reduced, but not significantly, in the DU group. The α-diversity was recovering at week six, and fully returned to baseline level at week 52. The β-diversity displayed significant changes at two weeks post treatment in both groups, mostly owing to a decreased mean relative abundance of *Bacteroidetes* and *Firmicutes*, and increased relative abundance of *Proteobacteria*, and all changes were largely restored by week six. These alterations transiently decreased the relative abundance of supposedly beneficial bacteria such as *Bacteroides*, *Faecalibacterium*, *Bifidobacterium*, *Phascolarctobacterium*, *Roseburia*, and *Blautia*, while permitting increased growth of potential pathogens such as *Escherichia/Shigella*, *Klebsiella* and *Streptococcus*. All changes returned to the baseline level by one year after the treatment showing that the antibiotic therapy caused short-term dysbiosis of the gut microbiota[27].

Another study[28] assessed the long-term changes after successful or failed *H. pylori* eradication therapy. 16S rRNA sequences of gastric biopsies samples from patients with successful and failed anti-*H. pylori* treatment revealed a significantly increase in relative abundance of 17 gastric genera after eradication, but only one genus decreased in abundance, *Helicobacter*. In contrast, in gastric biopsies of patients where eradication of *H. pylori* failed, no differentially distributed taxa were found. Likewise, the paired faecal samples from subjects with failed treatment showed no changes, while paired stool samples from successful eradication revealed changes in abundance of 21 taxa, 13 of which increased, including several Clostridia and *Bifidobacterium*, and eight decreased, mainly of the order *Bacteroidales*. The estimated Microbial Dysbiosis Index (MDI) significantly decreased after successful *H. pylori* eradication therapy but remained higher than that of *H. pylori*-negative controls. In contrast, there was no statistical difference between MDIs from before and after failed treatment[28].

ROLE OF PROBIOTICS IN ERADICATION THERAPY AND THEIR EFFECT ON GUT MICROBIOTA

The above studies emphasized a major problem: frequent failure of *H. pylori* eradication therapy. The use of probiotics, i.e., live microorganisms that confer a beneficial health effect to the host, as supplements during antibiotic therapy against *H. pylori* could increase the success rate, particularly in cases of multidrug resistant *H. pylori* strains[29]. Supplementing antibiotic treatment with probiotics can exert double benefits: a direct antagonistic effect on *H. pylori* and a balancing effect on dysbiosis of the microbiome. The most commonly used probiotic taxa belong to lactic acid bacteria of the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Enterococcus*, as well as *Bifidobacterium*[30,31].

Saracino et al[29] evaluated the direct bacteriostatic and bactericidal activity of five probiotic strains against *H. pylori*: the lactobacilli *L. casei*, *L. paracasei*, and *L. acidophilus*, *Bifidobacterium lactis* and *Streptococcus thermophilus*. Agar well diffusion and experimental time-kill curves showed both bacteriostatic and bactericidal activity of all probiotic strains, but the lactobacilli were the most effective in both tests, including against multidrug resistant *H. pylori* strains. Based on these encouraging data, the authors plan *in vivo* studies[29] to assess the effect of probiotics on eradication rates, and potentially also the mitigation of adverse events.

Cárdenas et al[32] explored clinical characteristics and faecal microbiota changes in patients in response to conventional *H. pylori* eradication therapy and to antibiotic therapy supplemented with the probiotic yeast *Saccharomyces boulardii.* This study reported significantly less frequent occurrence of associated gastrointestinal symptoms and showed increased bacterial α-diversity in the patients group that received *S. boulardii*. The authors concluded that the addition of *S. boulardii* to anti-*H. pylori* therapy decreased the frequency of side effects that could be related to accompanying changes in the gastrointestinal microbiota[32].

Supplementation of bismuth quadruple therapy with probiotics (Medilac-S, *Bacillus subtilis* and *Enterococcus faecium*) resulted in approximately 5% higher eradication efficacy, but the difference was not statistically significant[33]. Similar to a study discussed above[27], antibiotic therapy resulted in decreased α-diversity and a lower number of total OTUs by two weeks after treatment, which were restored to almost pre-treatment levels at six weeks[33]. However, the addition of Medilac-S did not affect the microbial diversity; there was no significant difference in α-diversity and total OTUs between the probiotic and the control group, even though supplementation with probiotics helped slightly to balance the gut microbiota after eradication therapy[33]. These data contradicted another study[34] in which addition of *E. faecium* as an adjuvant to triple therapy with vonoprazan as acid-inhibitory drug prevented the decrease in α-diversity after eradication therapy that was observed in the patient group without the supplement. The adjuvant also helped to mitigate side effects, although the observed trend was not statistically significant. A further study showed that supplementation with *E. faecium* and *Bifidobacterium animalis lactis* enhanced the *H. pylori* eradication rate in triple and quadruple therapies and significantly reduced adverse side effects[35], potentially by increasing the abundance of the probiotic taxa *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus,* and *Streptococcus*, in comparison to the group that received the conventional therapy.

A recent review[36] on the health promoting effects of lactic acid bacteria consumption summarized the effect of several lactobacilli on colonization, symptoms, clinical outcome, and eradication of various pathogens, including *H. pylori*. The reviewed clinical data as well as experiments in mice showed a moderately beneficial effect of the probiotic bacteria which belonged to the seven *Lactobacillus* species *L. salivarius*, *L. gasseri*, *L. johnsonii*, *L. casei*, *L. acidophilus*, *L. rhamnosus*, and *L. reuteri*. Overall, *H. pylori* colonization of the stomach was reduced, and gastric inflammation was alleviated. In addition, some of the reviewed papers showed an increased *H. pylori* eradication rate during antibiotic therapy when lactic acid bacteria were supplemented[36].

However, as another review[37] pointed out, published data on the effect of several probiotic strains are contradicting between studies, and there is no consensus as to which probiotic strains or formulations would be the most effective for different gastrointestinal problems. Despite some studies suggesting a positive effect of probiotics on the effectiveness of *H. pylori* eradication therapy, including those mentioned above, multiple other clinical studies produced negative results in that supplementation with probiotic showed no effect on the eradication rate[38-45], or even decreased the success rate of the antibiotic therapy[46]. Interestingly, even studies in which the same probiotics preparation was used yielded different results, either showing an increase in the eradication rate or no effect at all[47-49].

CONCLUDING REMARKS

In summary, at present, the potential benefit of supplementing triple and quadruple *H. pylori* antibiotics therapy with probiotics is still controversial. While some studies suggested potential benefits of probiotics on the eradication rate, many others found no effect. Even considering the potential effect in terms of reducing adverse events, the (very conservative) Maastricht V consensus stated that: "Only certain probiotics have been shown to be effective in reducing GI side effects caused by *H. pylori* eradication therapies. Specific strains should be chosen only upon the basis of a demonstrated clinical efficacy", and that "Certain probiotics may have a beneficial effect on *H. pylori* eradication"[25]. While supplementation with certain probiotics helped mitigating the adverse effects by lowering the frequency of gastrointestinal symptoms and by accelerating restoration of the microbiome, we are far from under-

standing the mechanisms involved. Further in-depth studies are required to explore the role of gut microbiota and their relation to *H. pylori*, and the mechanisms of the interaction of *H. pylori* and other bacteria of the stomach and duodenal mucosa, and probiotics. This may allow the prospective development of new approaches to improve the efficacy of *H. pylori* eradication therapy and to mitigate adverse effects associated with the disturbance of the gut microbiome. In future, it would be also of great interest to study the impact of infection with gastric non-pylori *Helicobacter* species on the gut microbiome. In this regard a novel detection method and database of multiple gastric *Helicobacter* species using MALDI-TOF has been recently developed, which is ready for application[50].

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2019; 7: 14.
2. Rajilic-Stojanovic M, Figueiredo C, Smet A, Hansen R, Kupcinskas J, Rokkas T, Andersen L, Machado JC, Ianiro G, Gasbarrini A, Leja M, Gisbert JP, Hold GL. Systematic review: gastric microbiota in health and disease. Aliment Pharmacol Ther 2020; 51: 582-602.
3. Ndegwa N, Ploner A, Andersson AF, Zagai U, Andreasson A, Vieth M, Talley NJ, Agreus L, Ye W. Gastric Microbiota in a Low-Helicobacter pylori Prevalence General Population and Their Associations With Gastric Lesions. Clin Transl Gastroenterol 2020; 11: e00191.
4. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, A DL, Wu F, Perez-Perez GI, Chen Y, Schweizer W, Zheng X, Contreras M, Dominguez-Bello MG, Blaser MJ. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med 2016; 8: 343ra382.
5. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1: 1311-1315.
6. Ozbey G, Sproston E, Hanafiah A. Helicobacter pylori Infection and Gastric Microbiota. Euroasian J Hepatogastroenterol 2020; 10: 36-41.
7. Saxena A, Mukhopadhyay AK, Nandi SP. Helicobacter pylori: Perturbation and restoration of gut microbiome. J Biosci 2020; 45: 110.
8. Cover TL, Blaser MJ. Helicobacter pylori in health and disease. Gastroenterology 2009; 136: 1863-1873.
9. Kamiya S, Backert S. Helicobacter pylori in human diseases. Cham, Switzerland: Springer: 2020.
10. Du Y, Zhu H, Liu J, Li J, Chang X, Zhou L, Chen M, Lu N, Li Z. Consensus on eradication of Helicobacter pylori and prevention and control of gastric cancer in China (2019, Shanghai). J Gastroenterol Hepatol 2020; 35: 624-629.
11. Mohammadi SO, Yadegar A, Kargar M, Mirjalali H, Kafilzadeh F. The impact of Helicobacter pylori infection on gut microbiota-endocrine system axis; modulation of metabolic hormone levels and energy homeostasis. J Diabetes Metab Disord 2020; 19: 1855-1861.
12. Ye Q, Shao X, Shen R, Chen D, Shen J. Changes in the human gut microbiota composition caused by Helicobacter pylori eradication therapy: A systematic review and meta-analysis. Helicobacter 2020; 25: e12713.
13. Zheng W, Miao J, Luo L, Long G, Chen B, Shu X, Gu W, Peng K, Li F, Zhao H, Botchway BOA, Fang M, Jiang M. The Effects of Helicobacter pylori Infection on Microbiota Associated With Gastric Mucosa and Immune Factors in Children. Front Immunol 2021; 12: 625586.
14. Miftahussurur M, Waskito LA, El-Serag HB, Ajami NJ, Nusi IA, Syam AF, Matsumoto T, Rezkitha YAA, Doohan D, Fauzia KA, Maimunah U, Sugihartono T, Uchida T, Yamaoka Y. Gastric microbiota and Helicobacter pylori in Indonesian population. Helicobacter 2020; 25: e12695.
15. Deng Y, Ding X, Song Q, Zhao G, Han L, Ding B, Wang X, Hao X, Li H. Alterations in mucosa-associated microbiota in the stomach of patients with gastric cancer. Cell Oncol (Dordr) 2021. doi: 10.1007/s13402-021-00596-y. Online ahead of print.
16. Gantuya B, El Serag HB, Matsumoto T, Ajami NJ, Uchida T, Oyuntsetseg K, Bolor D, Yamaoka Y. Gastric mucosal microbiota in a Mongolian population with gastric cancer and precursor conditions. Aliment Pharmacol Ther 2020; 51: 770-780.
17. Kadeerhan G, Gerhard M, Gao JJ, Mejias-Luque R, Zhang L, Vieth M, Ma JL, Bajbouj M, Suchanek S, Liu WD, Ulm K, Quante M, Li ZX, Zhou T, Schmid R, Classen M, Li WQ, Zhang Y, You WC, Pan KF. Microbiota alteration at different stages in gastric lesion progression: a population-based study in Linqu, China. Am J Cancer Res 2021; 11: 561-575.
18. Sarhadi V, Mathew B, Kokkola A, Karla T, Tikkanen M, Rautelin H, Lahti L, Puolakkainen P, Knuutila S. Gut microbiota of patients with different subtypes of gastric cancer and gastrointestinal stromal tumors. Gut Pathog 2021; 13: 11.
19. Spiegelhauer MR, Kupcinskas J, Johannesen TB, Urba M, Skieceviciene J, Jonaitis L, Frandsen TH, Kupcinskas L, Fuursted K, Andersen LP. Transient and Persistent Gastric Microbiome: Adherence of Bacteria in Gastric Cancer and Dyspeptic Patient Biopsies after Washing. J Clin Med 2020; 9: 1882.

20. Boehm ET, Thon C, Kupcinskas J, Steponaitiene R, Skieceviciene J, Canbay A, Malfertheiner P, Link A. Fusobacterium nucleatum is associated with worse prognosis in Lauren's diffuse type gastric cancer patients. Sci Rep 2020; 10: 16240.
21. Suarez-Jaramillo A, Baldeon ME, Prado B, Fornasini M, Cohen H, Flores N, Salvador I, Cargua O, Realpe J, Cardenas PA. Duodenal microbiome in patients with or without Helicobacter pylori infection. Helicobacter 2020; 25: e12753.
22. Kashiwagi S, Naito Y, Inoue R, Takagi T, Nakano T, Inada Y, Fukui A, Katada K, Mizushima K, Kamada K, Uchiyama K, Handa O, Ishikawa T, Itoh Y. Mucosa-Associated Microbiota in the Gastrointestinal Tract of Healthy Japanese Subjects. Digestion 2020; 101: 107-120.
23. Han HS, Lee SY, Oh SY, Moon HW, Cho H, Kim JH. Correlations of the Gastric and Duodenal Microbiota with Histological, Endoscopic, and Symptomatic Gastritis. J Clin Med 2019; 8: 312.
24. Wu ZF, Zou K, Xiang CJ, Jin ZJ, Ding HH, Xu S, Wu GN, Wang YH, Wu XY, Chen C, Yao XQ, Zhang JF, Liu FK. Helicobacter pylori infection is associated with the co-occurrence of bacteria in the oral cavity and the gastric mucosa. Helicobacter 2021; 26: e12786.
25. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM, European H, Microbiota Study G, Consensus p. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut 2017; 66: 6-30.
26. Kuo CJ, Lee CH, Chang ML, Lin CY, Lin WR, Su MY, Chiu CH, Tseng CN, Wu YS, Chiu CT, Lai CH. Multidrug resistance: The clinical dilemma of refractory Helicobacter pylori infection. J Microbiol Immunol Infect 2021: S1684-1182(21)00059-1.
27. Zhou Y, Ye Z, Lu J, Miao S, Lu X, Sun H, Wu J, Wang Y, Huang Y. Long-term changes in the gut microbiota after 14-day bismuth quadruple therapy in penicillin-allergic children. Helicobacter 2020; 25: e12721.
28. Guo Y, Zhang Y, Gerhard M, Gao JJ, Mejias-Luque R, Zhang L, Vieth M, Ma JL, Bajbouj M, Suchanek S, Liu WD, Ulm K, Quante M, Li ZX, Zhou T, Schmid R, Classen M, Li WQ, You WC, Pan KF. Effect of Helicobacter pylori on gastrointestinal microbiota: a population-based study in Linqu, a high-risk area of gastric cancer. Gut 2020; 69: 1598-1607.
29. Saracino IM, Pavoni M, Saccomanno L, Fiorini G, Pesci V, Foschi C, Piccirilli G, Bernardini G, Holton J, Figura N, Lazzarotto T, Borghi C, Vaira B. Antimicrobial Efficacy of Five Probiotic Strains Against Helicobacter pylori. Antibiotics (Basel) 2020; 9: 244.
30. Kamiya S, Yonezawa H, Osaki T. Role of Probiotics in Eradication Therapy for Helicobacter pylori Infection. Adv Exp Med Biol 2019; 1149: 243-255.
31. Quigley EMM. Prebiotics and Probiotics in Digestive Health. Clin Gastroenterol Hepatol 2019; 17: 333-344.
32. Cardenas PA, Garces D, Prado-Vivar B, Flores N, Fornasini M, Cohen H, Salvador I, Cargua O, Baldeon ME. Effect of Saccharomyces boulardii CNCM I-745 as complementary treatment of Helicobacter pylori infection on gut microbiome. Eur J Clin Microbiol Infect Dis 2020; 39: 1365-1372.
33. Tang B, Tang L, Huang C, Tian C, Chen L, He Z, Yang G, Zuo L, Zhao G, Liu E, Wang S, Lin H, He J, Yang S. The Effect of Probiotics Supplementation on Gut Microbiota After Helicobacter pylori Eradication: A Multicenter Randomized Controlled Trial. Infect Dis Ther 2021; 10: 317-333.
34. Kakiuchi T, Mizoe A, Yamamoto K, Imamura I, Hashiguchi K, Kawakubo H, Yamaguchi D, Fujioka Y, Nakayama A, Okuda M, Matsuo M. Effect of probiotics during vonoprazan-containing triple therapy on gut microbiota in Helicobacter pylori infection: A randomized controlled trial. Helicobacter 2020; 25: e12690.
35. Di Pierro F, Bertuccioli A, Saponara M, Ivaldi L. Impact of a two-bacterial-strain formula, containing Bifidobacterium animalis lactis BB-12 and Enterococcus faecium L3, administered before and after therapy for Helicobacter pylori eradication. Minerva Gastroenterol Dietol 2020; 66: 117-123.
36. Ren C, Faas MM, de Vos P. Disease managing capacities and mechanisms of host effects of lactic acid bacteria. Crit Rev Food Sci Nutr 2021; 61: 1365-1393.
37. Compare D, Sgamato C, Nardone OM, Rocco A, Coccoli P, Laurenza C, Nardone G. Probiotics in Gastrointestinal Diseases: All That Glitters Is Not Gold. Dig Dis 2021: Epub date 23 March 2021.
38. Nista EC, Candelli M, Cremonini F, Cazzato IA, Zocco MA, Franceschi F, Cammarota G, Gasbarrini G, Gasbarrini A. Bacillus clausii therapy to reduce side-effects of anti-Helicobacter pylori treatment: randomized, double-blind, placebo controlled trial. Aliment Pharmacol Ther 2004; 20: 1181-1188.
39. Tursi A, Brandimarte G, Giorgetti GM, Modeo ME. Effect of Lactobacillus casei supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure Helicobacter pylori infection. Med Sci Monit 2004; 10: CR662-666.
40. Tong JL, Ran ZH, Shen J, Zhang CX, Xiao SD. Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during Helicobacter pylori eradication therapy. Aliment Pharmacol Ther 2007; 25: 155-168.
41. Szajewska H, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of lactobacillus GG supplementation on Helicobacter pylori eradication rates and side effects during treatment in children. J Pediatr Gastroenterol Nutr 2009; 48: 431-436.
42. Manfredi M, Bizzarri B, Sacchero RI, Maccari S, Calabrese L, Fabbian F, De'Angelis GL. Helicobacter pylori infection in clinical practice: probiotics and a combination of probiotics + lactoferrin improve compliance, but not eradication, in sequential therapy. Helicobacter 2012; 17: 254-263.
43. Navarro-Rodriguez T, Silva FM, Barbuti RC, Mattar R, Moraes-Filho JP, de Oliveira MN, Bogsan CS, Chinzon D, Eisig JN. Association of a probiotic to a Helicobacter pylori eradication regimen does not increase efficacy or decreases the adverse effects of the treatment: a prospective, randomized, double-blind, placebo-controlled study. BMC Gastroenterol 2013; 13: 56.
44. Shavakhi A, Tabesh E, Yaghoutkar A, Hashemi H, Tabesh F, Khodadoostan M, Minakari M, Shavakhi S, Gholamrezaei A. The effects of multistrain probiotic compound on bismuth-containing quadruple therapy for Helicobacter pylori infection: a randomized placebo-controlled triple-blind study. Helicobacter 2013; 18: 280-284.

45. Dang Y, Reinhardt JD, Zhou X, Zhang G. The effect of probiotics supplementation on Helicobacter pylori eradication rates and side effects during eradication therapy: a meta-analysis. PLoS One 2014; 9: e111030.
46. Mirzaee V, Rezahosseini O. Randomized control trial: Comparison of Triple Therapy plus Probiotic Yogurt vs. Standard Triple Therapy on Helicobacter pylori Eradication. Iran Red Crescent Med J 2012; 14: 657-666.
47. Armuzzi A, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, Cammarota G, Anti M, De Lorenzo A, Pola P, Gasbarrini G, Gasbarrini A. The effect of oral administration of Lactobacillus GG on antibiotic-associated gastrointestinal side-effects during Helicobacter pylori eradication therapy. Aliment Pharmacol Ther 2001; 15: 163-169.
48. Armuzzi A, Cremonini F, Ojetti V, Bartolozzi F, Canducci F, Candelli M, Santarelli L, Cammarota G, De Lorenzo A, Pola P, Gasbarrini G, Gasbarrini A. Effect of Lactobacillus GG supplementation on antibiotic-associated gastrointestinal side effects during Helicobacter pylori eradication therapy: a pilot study. Digestion 2001; 63: 1-7.
49. Cremonini F, Di Caro S, Covino M, Armuzzi A, Gabrielli M, Santarelli L, Nista EC, Cammarota G, Gasbarrini G, Gasbarrini A. Effect of different probiotic preparations on anti-helicobacter pylori therapy-related side effects: a parallel group, triple blind, placebo-controlled study. Am J Gastroenterol 2002; 97: 2744-2749.
50. Berlamont H, De Witte C, De Bruyckere S, Fox JG, Backert S, Smet A, Boyen F, Haesebrouck F. Differentiation of Gastric Helicobacter Species Using MALDI-TOF Mass Spectrometry. Pathogens 2021; 10: 366.