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The Year in Helicobacter 2009

Guest Editors: Francis Megrand and Peter Malfertheiner
Helicobacter

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Guest Editors: Francis Mégraud and Peter Malfertheiner

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The Epidemiology of *Helicobacter pylori* and Public Health Implications

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Keywords

*H. pylori*, prevalence, transmission, risk factors, public health policy

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**Abstract**

This article presents a review of the literature on the epidemiology and public health implications of *Helicobacter pylori* infection published from April 2008 through to March 2009. The authors used MeSH terms “Helicobacter infections epidemiology,” “Helicobacter infections prevention and control” to search multiple databases (PubMed, Embase, Cochrane, Cochrane Library, EBMR, BIOSIS), and independently searched PubMed using the term “Helicobacter” with “Epidemiology,” “Transmission,” “Prevalence” or “Environment.” Articles without topical relevance were excluded. Two additional papers known to the authors were added. The identified literature is summarized by subtopic: reviews; prevalence; incidence; transmission; risk factors; and public health policy.

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**Reviews**

The search identified six review papers. Bruce and Maa-roos summarized studies on the epidemiology of *H. pylori* infection published in peer-reviewed journals [1]. Daugule and Rowland summarized articles on the epidemiology of *H. pylori* infection in children [2]. Tan et al. examined the changing *H. pylori* epidemiology in Asia [3]. All three of these reviews noted that the prevalence of *H. pylori* infection was decreasing globally. Goodman et al. reviewed studies of *H. pylori* infection in Canadian and related Arctic Aboriginal populations, revealing a relatively high prevalence of the infection and occurrence of associated disease in these groups [4]. Zhang et al. summarized 1986–2008 publications on re-infection, recurrence, or recrudescence of *H. pylori* identified in Medline, concluding that re-infection was not a major concern in clinical settings [5]. A review presenting Asia-Pacific consensus guidelines on gastric cancer prevention concluded that *H. pylori* screening and treatment strategies aimed at high-risk populations will probably reduce gastric cancer incidence and were therefore recommended [6].

**Prevalence**

The search identified 16 population-based prevalence studies from 12 countries, primarily from Asia and the Middle East [7–22]. Details from these studies grouped by *H. pylori* detection method are presented in Table 1. Among the noninvasive detection methods that are practical for population-based studies, the urea breath test (UBT) and stool antigen tests (SAT) are considered most accurate, while serology is the least costly and most widely available. *Helicobacter pylori* seroprevalence studies test most commonly for IgG which have the disadvantage of not differentiating current from past infection; whereas *H. pylori* IgG antibodies often decline to negative levels once the infection has resolved, the frequency and timing of this occurrence differs substantially across populations [23]. Furthermore, seronegativity is common in preschool-aged children.
demonstrated by other methods to have *H. pylori* infection [24]. One study from India used PCR on saliva and stool samples [11]; it has been noted that interpretation of PCR-based results is problematic [23]. One study of Japanese school children tested twice over a 12-month interval used a urine test for *H. pylori* IgG [15]. Urine tests for *H. pylori* infection are not widely used and information about their reliability is limited. Few population-based studies have used endoscopic procedures to evaluate gastrointestinal conditions; a study of adults

<table>
<thead>
<tr>
<th>First author</th>
<th>Detection method</th>
<th>Location/population</th>
<th>Subject selection</th>
<th>Age range (years)</th>
<th>Number tested</th>
<th>Prevalence</th>
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<tr>
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<td>Serum IgG and IgA</td>
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<td>1088</td>
<td>70.7</td>
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<tr>
<td>Kaya</td>
<td>Serum IgG</td>
<td>Turkey, asymptomatic people</td>
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<tr>
<td>Cheng</td>
<td>UBT</td>
<td>China, rural and urban</td>
<td>Not specified</td>
<td>2–79</td>
<td>1232</td>
<td>46.8</td>
</tr>
<tr>
<td>Tam</td>
<td>UBT</td>
<td>China, school children</td>
<td>Random sampling from schools</td>
<td>6–19</td>
<td>2480</td>
<td>13.1</td>
</tr>
<tr>
<td>Mohammad</td>
<td>UBT</td>
<td>Egypt</td>
<td>Not specified</td>
<td>6–15</td>
<td>286</td>
<td>72.4</td>
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<tr>
<td>Kori</td>
<td>SAT</td>
<td>Israel, daycare children</td>
<td>Not specified</td>
<td>0.25–5</td>
<td>316</td>
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<tr>
<td>Cherian</td>
<td>SAT</td>
<td>Australia, African refugees</td>
<td>All presenting for health assessment during study period</td>
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<td>82</td>
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<tr>
<td>Yücel</td>
<td>SAT</td>
<td>Turkey, university students</td>
<td>Random sampling from unspecified number of volunteers</td>
<td>Mean = 21b</td>
<td>200</td>
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<tr>
<td>Shi</td>
<td>Serum IgG and UBT</td>
<td>China, rural</td>
<td>Cluster sampling</td>
<td>5–100</td>
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<td>62.1c</td>
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<td>Zagari</td>
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<td>Italy, residents of two northern villages</td>
<td>Recruited from participants in earlier population-based survey</td>
<td>≥32 (mean = 59d)</td>
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<td>58</td>
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<tr>
<td>Mishra</td>
<td>PCR on saliva and stool</td>
<td>India, university employee families and urban slum dwellers</td>
<td>Not specified</td>
<td>0.67–60</td>
<td>245</td>
<td>45.7 (saliva) 42.8 (stool)</td>
</tr>
<tr>
<td>Naito</td>
<td>Urinary IgG</td>
<td>Japan, Tokyo school children</td>
<td>Not specified</td>
<td>4</td>
<td>452</td>
<td>5.3 (time 1)e 6.7 (time 2) 4.7 (time 1) 4.0 (time 2) 4.0 (time 1) 4.6 (time 2)</td>
</tr>
</tbody>
</table>

aNot specified if prevalence was based on positivity on one or both tests.
bRange not specified.
cPrevalence based on positivity on one or both tests.
dPositivity defined by positive on at least two of UBT, histology, rapid urease test.
eTimes 1 and 2 were approximately 12 months apart.
from two villages in northern Italy classified *H. pylori* status using the UBT and evaluation of gastric biopsies [22].

The reported prevalence ranged from 4% in Japanese children to 82% in African refugee children in Australia [8,15]. A prevalence of 15% or lower was reported for Australian lab patients, Malaysian blood donors, and Chinese and Japanese school children [14,15,17,20]. A prevalence of 24–25% was reported for Israeli children attending daycare centers and unspecified individuals from Turkey [10,21]. Among the Italian villagers (mean age = 59 years), the prevalence was 58%, considerably higher than the 34% observed in an earlier similar study of adults in northern Swedish communities (mean age = 52 years) [25]. A prevalence of 60% or more was reported for groups in Albania, Egypt, Iran, Turkey, and China [7,9,12,13,16,18].

### Incidence

Only three studies examined rates of onset of new *H. pylori* infections or reinfections. A Bangladeshi study examined new infections from birth to 2 years of age in 258 children [26]. They observed that few children (number not reported but less than 15% by serum IgG, IgA and/or SAT) showed evidence of infection at 6 months of age, but by 2 years positivity was 49% by SAT and around 60% (number not reported) by IgG and/or IgA. The Japanese study that used urine tests at two time points examined rates of acquisition and loss of infection in 452 children [15]. They reported that the 12-month incidence decreased with age, 2.6% among 4-year-olds, 1.3% among 7-year-olds, and 0.65% among 10-year-olds, while rates of apparent infection loss were 1.3%, 2%, and 0 in the 4-, 7- and 10-year-old groups, respectively. Statistical precision for the age-specific incidence comparisons was not reported. An Israeli study examined rates of new infection in adult dyspeptic patients (*n* = 39) who had a negative *H. pylori* test 7 years earlier and re-infection in adult patients (*n* = 26) after successful *H. pylori* therapy [27]. One patient in each group had a positive UBT; however, the small sample makes estimates of incidence rates highly imprecise.

### Transmission

The high number of papers published on *H. pylori* transmission during the last year reveal the information gaps and inconsistent results that continue to hamper our understanding of how this organism spreads. Intrafamilial transmission, by direct person-to-person contact, has long been thought to be a major mode of transmission [28,29]. Weyermann et al. tried to determine the independent contribution of mothers, fathers, and siblings to acquisition of *H. pylori* during childhood in a German population [30]. *Helicobacter pylori* status was based on 13C-UBT and/or monoclonal SAT performed on stool samples, which lack information on the genetic similarity of strains from different family members. Adjusting for the siblings’ and father’s *H. pylori* status, the odds ratio (OR) for the effect on the index child of the mother being infected was 13 (95% confidence interval (CI), 3–55). The authors extracted data from similar studies to estimate the odds of infection adjusted for the infection status of other family members, concluding that after adjustment for maternal infection status, the OR among children for having an infected sibling or father decreased substantially in the German population and others. It should be noted that siblings’ infection status appears to matter in populations where large families are common [31], and that the mother’s infection status may be more strongly associated than other family members with household hygiene and other risk factors.

Using genotyping methods, assessments of the relatedness of family members’ *H. pylori* strains were performed in Japan, Bangladesh, and Peru [32–34]. Child–mother strain pairs matched for 69, 46, and 30% of the studied populations, respectively. As with studies of family members’ general infection status, these results suggest varying contributions of mother-to-child (and overall intra-familial) transmission in developing and developed countries, as a consequence of different living conditions and age-specific infection patterns. In a larger study that estimated the similarity of sequences in populations of South Africa, the United Kingdom, the United States, Korea, and Colombia, the conclusion was similar, and variability of strains within families was higher in rural than in urban areas [35]. As the authors pointed out, *H. pylori* research relying on genotyping would benefit from more thorough attempts to identify multiple strains in individuals, which would be of particular relevance for better understanding modes of transmission.

Direct person-to-person transmission may occur via the oral–oral, fecal–oral, or gastro-oral route. Burgers et al. tested for the concurrent presence of *H. pylori* in the mouth and stomach of 94 individuals [36]. *Helicobacter pylori* was detected in the oral cavities of 17% of gastric biopsy patients, some of whom did not show evidence of stomach colonization. However, the presence of organisms in a particular location is not clear evidence that transmission commonly occurs via the corresponding pathway. Tonsils have been proposed as an extra-gastroduodenal reservoir for the bacterium,
but one study observed that tonsil removal did not appear to affect the risk of *H. pylori* transmission [37].

The role of external reservoirs in *H. pylori* transmission has not been ruled out, particularly in rural and developing areas [38]. Water has been one of the most well-studied ecosystems for *H. pylori* survival outside the human digestive tract. A Japanese study compared *H. pylori* prevalence in three populations with different drinking water sources (two with river water, one with groundwater) [39]; while the population with the groundwater source had a much lower prevalence, the small numbers in this ecologic comparison limit its value. Other studies attempting to identify *H. pylori* DNA in water provide conflicting evidence [40,41]. In Mexico, Mazari-Hiriart et al. detected the 16S rRNA and *cagA* genes of *H. pylori* in 44% and 14%, respectively, of samples from ground and surface water [41]. In contrast, Bockelman et al. were unable to detect the *H. pylori* 16S rRNA gene in samples from artificial recharge systems in Spain, Italy, and Belgium [40]. The inconsistent results may reflect different water treatment modalities and/or variations in PCR procedures (e.g. DNA isolation methods, primer sequences, and application of nested or quantitative-PCR). Research in this area would benefit from identification of the optimal techniques for reliable assessment of the presence or absence of *H. pylori* DNA in suspected environmental reservoirs. It should be noted, however, that demonstration of DNA in a potential environmental reservoir is not clear evidence of the transmissibility of the organisms, which may or may not be viable. Culture of *H. pylori* organisms from these sources would provide stronger evidence.

From one of the few studies based on culture of *H. pylori* outside the human digestive tract, Cellini et al. characterized one strain found in marine zooplankton [42]. This environmental strain was able to form biofilms in a more structured way than clinical strains. Biofilms are a possible microenvironment where *H. pylori* may subsist in water systems, and another study showed that a cultured strain of clinical origin incorporated in mature, multispecies biofilms formed in a model reactor simulating unchlorinated drinking water distribution systems [43]. Another microenvironment that may promote *H. pylori* survival in water is the intracellular habitat of protozoa. However, when studying the spatial distribution of *Helicobacter* spp. and *Acanthamoeba* in river water samples, Kawaguchi et al. did not detect a clear association between the two microorganisms [44].

Two other possible extra-human reservoirs assessed this year were food and the digestive tract of animals [45,46]. When testing raw milk for the presence of the *H. pylori* glmM gene, Quaglia et al. were able to amplify glmM in 34.7% of the samples using a nested-PCR approach [46]. Ghil et al. assessed the prevalence of *Helicobacter* spp. in feces and saliva from cats in Korea [45]. Despite detecting the presence of *Helicobacter* spp. in 77.6% of the cats using genus-specific primers, all species-specific PCR for *H. pylori* were negative. As long as there is no consensus on the reliability of particular PCR methods and a valid assessment of the physiologic status of *H. pylori* found in these environments, it is not possible to clarify the role of particular external reservoirs in *H. pylori* transmission.

**Risk Factors**

Most reports on risk factors focused on socioeconomic indicators. Most of the studies examined cross-sectional associations between exposures of interest and being infected at the time of screening, which cannot differentiate determinants of acquisition from determinants of persistent infection. Among Israeli children in day care, low socioeconomic status was associated with *H. pylori* infection [10]; this study collected data on family size, residential crowding, parent’s education, and country of birth, but the basis for classifying low status was not specified. Among Egyptian children, *H. pylori* prevalence was highest in children attending school in deprived areas [12]; residents of Cairo had the highest prevalence among the locations studied. A study of mainly university employees in India demonstrated a relationship between living in semi-urban slums and *H. pylori* status classified by PCR-based stool and saliva tests [11]; other factors were not controlled in this comparison. A Chinese study of 2480 school-aged children identified an association with lack of formal education of the mother, and an Iranian study of 851 individuals found low education of the mother, father, and subject to be associated with *H. pylori* infection [16,20]. A study of Turkish university students observed little relationship to *H. pylori* status of parents’ education level, number of family members, and income level [21]. The authors noted that selection of youth who were mainly from state dormitories, which is determined by parent’s income level, was a limitation of their study. A Chinese study of 1457 individuals identified associations with low education, low family income, and not cleaning a cup after use [18].

A few reports in addition to the Israeli and Turkish studies examined family size. Two studies of child populations observed in multivariable analyses that a household size greater than five was associated with *H. pylori* infection [19,20]. Two studies of adult populations also observed that household size greater than five during childhood was associated with *H. pylori* infection when other factors were not controlled, but this...
relationship did not appear independent of other factors in multivariable analyses [16,18].

Two studies examined occupational exposures that increase the risk of infection. In a Belgian study, 587 employees of institutions for children with intellectual disabilities were compared with 390 employees of companies that do not serve children [47]. Assessed H. pylori risk factors included parent’s education level, number of household members during childhood, number of children sharing a room in childhood, and travel to tropical regions along with occupational exposures, such as personal contact, fecal contact, and washing and feeding of inhabitants. In a multivariable logistic regression model, only fecal contact was associated with a clear increase in the odds of H. pylori infection (OR, 4.0; 95% CI 1.7–9.5). A study of Swiss workers used a prospective cohort design to examine incidence of H. pylori IgG and IgA seroconversion in relation to sewage exposure [48]; 332 workers exposed to sewage and 446 nonexposed workers were tested at baseline and five time points at approximately 1 year intervals. Using seroconversion as an endpoint for survival analysis, no clear effect of exposure to sewage was observed, when controlling for education level, nationality, country of childhood, smoking, and alcohol intake.

The Tunisian study of 1055 first grade students identified bed sharing and bottle weaning after 18 months as risk factors [19]. The Turkish study observed little relationship to H. pylori infection of various hygiene practices or smoking, alcohol, coffee or tea consumption [21]. A study of 1391 Albanian individuals did not collect data on number in household, and in a multivariable analysis only female gender and age greater than 40 were associated with H. pylori seropositivity [13]. Among African refugee children in Australia, ethnicity, country of transit and pre-migration anti-malarial treatment were associated with H. pylori infection, but in a multivariable logistic regression model, only pre-migration anti-malarial treatment appeared to retain an independent association, in the direction of reduced odds of infection [8].

### Public Health Policy

Evidence from epidemiologic research provides the basis for disease control and prevention policy. In particular, the identification of high-prevalence populations helps identify target communities for cost-effective interventions, and the identification of modifiable risk factors yields potentially effective interventions. While the epidemiologic research on H. pylori has gone a long way toward identifying high-prevalence communities around the world, including some within countries where average prevalence is low, little work has been done on interventions aimed at interrupting transmission (i.e. primary prevention of H. pylori infection), and the search for this review identified no such reports. A modest amount of prevention research has focused on H. pylori infection as a modifiable risk factor for associated digestive diseases (i.e. tertiary prevention of complications from H. pylori infection), including four reports published in the last year on screening and treatment strategies in targeted populations.

A community-based H. pylori screening and treatment program in Denmark randomized 20,011 40 to 64-year-old residents of Odense identified by civil registration number to H. pylori screening and treatment (screened group) or no intervention [49]. After 5 years, investigators estimated the effect of the program on rates of dyspepsia and peptic ulcers, drug use, doctor visits, and health related-quality of life. The most noteworthy benefit of the program was a 33% lower incidence of peptic ulcers. A modest reduction in dyspepsia was observed in the screened group, but this was similar in magnitude to an unexplained excess prevalence of dyspepsia in the screened group at baseline. The authors reported that the cost of dyspepsia-related health care was lower in the screened group, but the savings were exceeded by the cost of screening and treatment. However, the authors did not estimate the cost-effectiveness of the program for disease prevention, for example, the cost per peptic ulcer prevented. While the intervention was not cost saving, data in the report suggest that the total expenditure could be considered reasonable for the number of peptic ulcer cases prevented.

Another study examined the cost-effectiveness of H. pylori treatment in H. pylori-positive long-term proton pump inhibitor (PPI) users in the UK randomly assigned to anti-H. pylori therapy (n = 93) or placebo (n = 91) [50]. After 2 years, the treatment group had substantially fewer prescriptions, GP consultations and GI-related home visits, upper endoscopies, abdominal/pelvic ultrasound scans, and dyspepsia symptoms, although heartburn symptoms increased. The average cost savings per patient during 2 years after subtracting the cost of screening and treatment was £93. The authors concluded that H. pylori treatment in long-term PPI users is an economically dominant strategy that reduces healthcare costs and symptom severity.

Two studies estimated the cost-effectiveness of population-based H. pylori screening and treatment for gastric cancer prevention, in a high-risk region of China and the male Chinese population of Singapore [51,52]. Xie et al. assessed one-time screening and treatment with either serology or UBT in terms of cost per gastric cancer case prevented, life-year saved and quality-adjusted life year gained [52]. Yeh et al. assessed
one-time screening with serology and treatment, as well as strategies of re-screening of seronegatives and universal treatment without screening in terms of gastric cancer risk reduction and cost per year of life saved [51]. Both analyses showed that the evaluated strategies were reasonably cost-effective compared to no intervention, and that this conclusion is robust to reasonable ranges for uncertain input values pertaining to factors such as screening method accuracy, treatment success rates, the fraction of gastric cancer prevented by \textit{H. pylori} elimination, and the optimal target age group (e.g. before precancerous lesions typically develop). Emerging evidence from gastric cancer prevention trials is revealing more about the latter two factors [6].

These studies strengthen a growing body of evidence that most \textit{H. pylori} screening and treatment strategies considered are cost-effective for prevention of \textit{H. pylori}-associated disease in most population studied. They are less compelling, however, for comparing alternative strategies, particularly in light of uncertain inputs. Future research in this area should focus on whether it is worth the incremental cost to save additional lives with more costly strategies such as using the UBT rather than serology for screening, screening more than once, or targeting younger populations, and should identify the variables to which such choices are sensitive.

Conflicts of Interest

The authors have declared no conflicts of interest.

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1 Bruce MG, Maaroor H. Epidemiology of \textit{Helicobacter pylori} infection. \textit{Helicobacter} 2008;13(Suppl. 1):1–6.
Diagnosis of Helicobacter pylori Infection

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Keywords
Urease test, histology, culture, stool antigen test, urea breath test, molecular methods, antimicrobial susceptibility testing.

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Abstract
The articles published this last year in the field of Helicobacter pylori diagnosis reported the development of in vivo histology, small improvements in some invasive methods (urease test, culture, and histology) and new kits for the stool antigen tests. They also contributed to increasing our knowledge, by further exploration into specific conditions for the urea breath test and into the significance of cagA antibodies. The role of serum markers of atrophy was also confirmed. Molecular methods are still being developed for direct genotyping, detection of H. pylori and its clarithromycin resistance, either by polymerase chain reaction or fluorescent in-situ hybridization. For the first time, there was a report on a possible interest of magnetic resonance spectroscopy.

A variety of tests for detecting Helicobacter pylori infection since the discovery of this pathogen have been described. While there has been no recent breakthrough in this topic, a number of original articles coming especially from emerging countries were published last year on the different molecular and nonmolecular diagnostic tests for H. pylori.

Non-molecular Methods

Invasive Tests

Graham et al. published a review article providing recommendations regarding when endoscopic gastric mucosa assessment must be preferred rather than non-invasive methods [1].

Endoscopy

To obtain biopsies, an upper digestive endoscopy must be performed. Cho et al. proposed a new method of standard endoscopic diagnosis of H. pylori: the phenol red mucosal pH test. A 0.1% phenol red solution was sprayed on the gastric mucosa. The extent of staining, expressed as a staining score, was positively correlated with the urea breath test (UBT) values and with H. pylori density as measured by histology. The pH measured in this study with an antimony electrode was significantly higher in H. pylori infected mucosa. Therefore, endoscopic phenol red staining may be an alternative method for the diagnosis of H. pylori infection [2].

The new methods of magnifying endoscopy currently developed have an added value compared to standard endoscopy, i.e. the possibility of performing in vivo histology. A prospective study on 129 patients performed in Turkey confirmed that the new method of high resolution magnifying endoscopy is superior to standard endoscopy for the diagnosis of H. pylori gastritis, and identification of specific histopathologic features, such as atrophy and intestinal metaplasia seems possible [3]. In a review of confocal laser endomicroscopy, Kiesslich et al. highlighted the possibility of virtual histology and its role in diagnosing H. pylori gastritis and targeting biopsy specimens [4].

Interestingly, Kim et al. studied the sites for performing biopsies to detect H. pylori in 194 patients with gastric cancer. They found that the best site was the upper body greater curvature (sensitivity of histology: 95.1%; 95% CI: 89.6–98.2), probably due to the proportion of atrophy and intestinal metaplasia, which were significantly lower than in the antrum and in the upper body lesser curvature which were also tested [5].

Histology

An alternative to Giemsa staining has been proposed in Thailand [6]. A mixture of carbol fuschin and alcian blue staining was compared blindly to Giemsa and hematoxylin & eosin staining on 423 histologic
preparations. They found the same rate of positive samples and highlighted the low cost and simplicity of the combined staining and its value in identifying goblet cells in intestinal metaplasia.

The impact of mixed H. pylori infections was studied in 30 patients by Sheu et al. [7]. They found that the seven patients with mixed infections had marginally higher scores of chronic inflammation and H. pylori density in the corpus and higher rates of intestinal metaplasia in the antrum ($p = .005$) compared to the 23 patients with a single strain.

An article referred to the new staging system for atrophy (OLGA) and its application in diagnostic practice [8]. It was also used to assess atrophic gastritis in 63 H. pylori positive patients with various gastric diseases. They found the OLGA staging system useful for the assessment of the severity of atrophic gastritis and simple to use [9]. In another study concerning different risks of gastric cancer in populations, the OLGA staging mirrored the gastric cancer incidence [10].

The histogenesis of gastric carcinomas was re-evaluated by Kakinoki et al. [11]. They compared H. pylori-negative and -positive cases and found that carcinoma cells could occur independently of the intestinal metaplasia. If this finding is confirmed, it would indicate that intestinal metaplasia is not a precancerous but a paracancerous lesion.

Urease test

As in previous studies, the sensitivity of the rapid urease test (RUT) was shown to be reduced in patients with bleeding ulcers but the short-term use of standard dose proton-pump inhibitor did not have an impact [12].

As a solution to the low sensitivity of the RUT, some authors proposed to increase the number of biopsies tested up to four. Comparing one biopsy to four, the positive results increased from 52 to 96%, respectively [13].

Culture

Because of the slow growth and the particular requirements of H. pylori with regard to culture conditions, this area still remains a particular challenge. Sainsus et al. tried to develop a liquid culture medium for the rapid isolation, identification, and subsequent antibiotic susceptibility testing of H. pylori from biopsy specimens. They selected Ham’s F12 medium with 5% horse serum with antibiotics which provided the most rapid and reliable growth. The CIM medium seems a promising solution to solve some of the current problems concerning H. pylori culture in solid media [14].

Non-invasive Tests

Urea breath test

$^{13}$C-UBT has been shown numerous times to be the most accurate H. pylori diagnostic test. Several remaining questions were addressed this year. Buzas and Szeles compared UBT values after first-, second- and third-line treatments. They found as in previous studies that higher pretreatment UBT values were associated with lower eradication rates but interestingly, they also showed a marked tendency to increase UBT values for the patients who failed, i.e. from $13.2\%$ (CI: 7.3–19.1) to $19.2\%$ (CI: 13.4–25.0) after second-line therapy and to $25.8\%$ (CI: 19.8–31.2) after third-line therapy, but they could not explain this phenomenon [15]. In another study, UBT values were also found to be higher when clarithromycin resistant H. pylori were present, but not when other resistances occurred [16].

The possibility of false positive results due to urease positive bacteria from the oral cavity in patients with atrophic gastritis was highlighted by Osaki et al. indicating that the histologic status of the stomach, i.e. presence or absence of atrophy, must be considered in interpreting the results [17]. To avoid false positive results, the capsule UBT can be used [18].

There is another situation where false positive results may occur, i.e. in children younger than 6 years of age. The cause may not only be the effect of endogenous CO$_2$ production but also other unidentified factors [19]. In contrast to the RUT, the UBT was found to be unreliable in patients with Billroth II gastrectomy [20]. Testing for the eradication of H. pylori is an important aspect of clinical trial design and is of critical importance in the evaluation of new therapies for this pathogen. From the evaluation of Vakil et al. a single UBT, 4 weeks after treatment was as effective as two serial breath tests in confirming H. pylori eradication and the incremental cost of the second breath test was very high with no incremental clinical benefit [21]. A study performed in Israel to evaluate the indications for UBT used by primary care doctors and to examine the appropriateness of these indications to the accepted guidelines of the Maastricht Consensus Report revealed a substantial noncompliance with guidelines for H. pylori testing among primary care doctors in this country [22].

Stool antigen test

Stool antigen detection kits for the diagnosis of H. pylori infection have been widely used because of their full noninvasive nature. Mohammadian et al. presented a simple, rapid, and cost-effective production of a
polyclonal antibody against alkyl hydroperoxide reductase (AhpC) of *H. pylori* for stool-antigen enzyme immunoassay [23]. Nguyen et al. evaluated the sensitivity and specificity of the new monoclonal antibody-based antigen-in-stool enzyme immunoassay (Premier Platinum HpSA PLUS; Meridian Bioscience, Cincinnati, OH, USA) for diagnosis of *H. pylori* infection in Vietnamese children. The sensitivity was 96.6% (95% CI: 93.3–98.5) and the specificity 94.9% (95% CI: 88.5–98.3) [24]. In addition, Kuloğlu et al. evaluated the diagnostic accuracy of a rapid immunochromatographic stool antigen test (Rapid HpSA; LINEAR Chemical, Barcelona, Spain) and a practical low-dose 14C-UBT (Heliprobe™; Kibion, Uppsala, Sweden) in children before and after eradication therapy. The sensitivity of Rapid HpSA and 14C-UBT was 65% and 92.5% (*p* = .0003), respectively; the specificity of Rapid HpSA and 14C-UBT was 92.3 and 85.5% (*p* = .180), respectively. After eradication therapy, endoscopy, 14C-UBT and Rapid HpSA were repeated. Both tests had the same specificity (100%) while the sensitivity of Rapid HpSA and 14C-UBT was 60 and 100%, respectively [25]. However, the most interesting study compared six tests and the results are reported in Table 1 [26].

### Table 1 Summary of sensitivity and specificity of the stool antigen tests cited by Blanco et al. [26]

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunodiagnostics ELISA</td>
<td>87.3</td>
<td>83.3</td>
</tr>
<tr>
<td>HpsStAR</td>
<td>95</td>
<td>66.6</td>
</tr>
<tr>
<td>HpsA/EIA</td>
<td>92.5</td>
<td>72.2</td>
</tr>
<tr>
<td><em>H. pylori</em> Lihtest</td>
<td>83.6</td>
<td>66.6</td>
</tr>
<tr>
<td>Immunolab HpSA</td>
<td>52.5</td>
<td>94.4</td>
</tr>
<tr>
<td>RAPID HpStAR</td>
<td>78.8</td>
<td>55.5</td>
</tr>
</tbody>
</table>

Anti-CagA antibodies (detected either by a specific ELISA or immunoblot) have been described as long standing antibodies of interest to confirm *H. pylori* etiology years after the disappearance of the bacterium. Recently, Veijola et al. confirmed that CagA antibodies detected by immunoblot (Helico Blot 2.1) could still be detected in 87% of the patients 10 years or more after a successful *H. pylori* eradication [30]. Studies were performed in several countries with various results. In Australia, the use of Helicoblot 2.1 detecting CagA antibodies improved the sensitivity of *H. pylori* detection in patients with noncardia gastric cancer from 79% with *H. pylori* ELISA to 94%. Interestingly, pepsinogen I levels showed the lowest median level to be in cases which were negative by ELISA but positive by immunoblot [31]. In Mexico, CagA antibodies were associated with young gastric cancer cases only, and were also a risk factor for intestinal metaplasia [32]. In Estonia, positivity for CagA antibodies was able to predict the development of atrophy, particularly in the corpus (OR: 7.0, 1.8–27.7). In addition, the prevalence of canalicular antibodies increased with the duration of the *H. pylori* gastritis (22–46% in 12 years) [33]. In contrast, in India, the prevalence of *H. pylori* detected by *H. pylori* serology or CagA antibodies was comparable both in gastric cancer patients and in controls [34].
Serum biomarkers, especially those proposed by BioHit in the GastroPanel (pepsinogen I & II, gastrin 17, H. pylori Ab) have been tested in comparison to histology. In the Kalixanda study (Northern Sweden), an overall agreement of 96% was found. Sensitivity and specificity of the markers for atrophic gastritis were 71 and 98%, respectively [35]. The GastroPanel was also able to differentiate Japanese patients into two groups with “healthy” or “diseased” stomachs with a 94% accuracy. In total, 5% of the patients had advanced atrophic corpus gastritis [36].

With regard to cost effectiveness, a Markov model was designed based on 237,900 Chinese males, aged 35–44 years and living in Singapore, who would be screened and treated with eradication therapy. Serology appeared to be twice as cost effective as the UBT in screening this population [37].

From two studies concerning the value of RAPIRUN test, a urinary antibody test. Nguyen et al. verified that in the Vietnamese population sensitivity, specificity, and accuracy of the test were 79.5, 90.7, and 84.5%, respectively [38]. These values are in accordance with the overview presented by Yamamoto et al. concerning the diagnosis of H. pylori infection using the same detection kit [39]. The development of a model for economic evaluation related to the diagnosis accuracy of near patient tests used in office laboratories, as opposed to using hospital-based tests was presented by Fauli et al. [40].

### Molecular Methods

#### Detection

Over the last year, novel molecular methods based mainly on a real-time polymerase chain reaction (PCR) have been described to improve the detection and characterization of H. pylori. A new multiplex fluorescence resonance energy transfer real-time PCR, for amplification of H. pylori ureA and human β-globin, was developed, allowing quantification of the bacterial density by determination of the ureA/β-globin amount ratio in gastric biopsies. Using this assay, a significantly increased bacterial density was found in macroscopic erosions when compared with the healthy portion of the stomach (p < .01). This PCR was not able to detect the ureA gene in H. pylori-positive formalin-fixed paraffin-embedded biopsy sections, probably because DNA was broken and amplification of a 411-bp fragment was not possible [41]. To overcome the problem of extensive genetic polymorphism for precise PCR detection of H. pylori, Liu et al. developed a novel molecular approach, based on real-time reverse transcriptase (RT)-PCR and in situ hybridization, targeting a 76-bp region of H. pylori 16S rRNA, that is highly conserved in a large number of H. pylori strains and is specific to this bacterium. Both approaches were shown to be very sensitive and specific and combined together can be used for specific detection, identification and quantification of H. pylori in biological samples, from humans, animals, or environmental source [42]. In another study, real-time PCR targeting the 23S rRNA gene applied on aortic and left internal mammary artery biopsies, was used to demonstrate, for the first time, that acute coronary ischemia was significantly more prevalent in H. pylori-positive patients than in H. pylori-negative patients (p = .001), suggesting a pathogenic role of this bacterium in atherosclerosis [43]. Finally, Gill et al. developed a nanodiagnostic method using thermophilic helicase-dependent isothermal amplification of ureC and gold nanoparticle probes for hybridization and colorimetric detection of H. pylori DNA. This method allowed the detection of 10 CFU/mL within less than 1 hour and provided a sensitivity of 92.5% and a specificity of 95.4%, with culture as the reference [44].

Sugimoto et al. identified 26 sets of primers used to detect H. pylori. They first tested their detection limit. Five of the 26 sets with a detection limit <100 CFU/mL were then tested further. All produced some false positive results. These results indicate that results of H. pylori detection by PCR outside of the stomach should be interpreted with caution. Identification based on the presence of multiple specific genes could be the way forward [45].

#### Genotyping

Simultaneous genotyping of bacterial and host cells is sometimes difficult due to the small amounts of sample. To overcome this problem, Ryberg et al. used the multiple displacement amplification technique on minute amounts of gastric biopsy specimens. Then, the amplified DNA was used for concurrent PCR-based genotyping, of both H. pylori 16S rRNA, vacA, hsp60, ureI, sod, ureA and cagA genes, and human cytokine polymorphisms [46]. Puz et al. developed a novel noninvasive genotyping method, using stool specimens, based on two H. pylori-specific biprobe real-time PCR assays targeting the glmM and recA genes. Discrimination between strains is made using the differences in the melting temperature of the amplicons. The sensitivity of the assay on stool samples was 92.2% and the specificity was 100%. A discriminatory capacity of 100% was achieved when the sequence analysis of the glmM amplicon was performed. Due to its noninvasiveness and high accuracy in detection and discrimination of...
H. pylori strains, this genotyping assay has the potential of being used in large-scale studies, contributing to the clarification of the transmission pathways of this organism [47]. Paraffin-embedded gastric biopsies were used for H. pylori genotyping with success (97% of the cases). The genotyping enabled a comparison of the prevalence of cagA positive, vacA s1m1 genotypes in high and low risk areas for gastric cancer in Colombia (84.3 and 60.5%, respectively) [48]. Kanada et al. evaluated the accuracy of immunohistochemistry for genotyping the cagA East-Asian EPIYA motif. They used a monoclonal antibody against this motif and tested it on gastric biopsy samples from Japanese patients. Compared to sequencing of the cagA 3′-region, containing the EPIYA motifs, the new assay showed a sensitivity of 93.2% and a specificity of 72.7%, suggesting a further optimization to be useful as a cagA typing method [49].

Antimicrobial Resistance

With the growing H. pylori resistance to antibiotics, molecular diagnostic tests for an accurate and rapid identification of these strains are an attractive alternative to the time consuming culture-based susceptibility testing. Thus, this is still an emergent field in H. pylori research, targeting in particular the clarithromycin resistance-associated gene mutations. A study employing Scorpion primers, which combine a primer and a probe in a single molecule, was reported by Burucoa et al. to detect H. pylori and clarithromycin resistance directly on gastric biopsies. The Scorpion PCR was highly sensitive and specific (98.3% and 92.5%) in detecting H. pylori, using culture as the gold standard, and, as usual, it was more sensitive in detecting mixed populations with susceptible and resistant strains than the E-test. Clarithromycin genotypes determined with Scorpion PCR were concordant with those obtained by PCR-restriction fragment length polymorphism [50]. Fluorescent in-situ hybridization (FISH) can be used to identify H. pylori and antibiotic resistance in biopsy specimens without PCR. Tajbakhsh et al. reported a FISH procedure targeting the H. pylori ribosomal RNA, and this assay showed a sensitivity and specificity of 97.9 and 100% respectively, for detection of H. pylori, when compared to histology. However, the FISH assay, employing four different probes, one wild type and three mutated, showed a weak sensitivity in detecting clarithromycin susceptibility-associated genotypes, as only 19 of the 47 FISH-positive samples were recognized with these probes [51]. Another study employing the FISH technology, performed using DNA ProbeMix targeting the 16S rRNA H. pylori gene, showed a satisfactory sensitivity on both fresh and paraffin-embedded biopsies isolated from children (84.1 and 80.7%, respectively), previously diagnosed as H. pylori positive by histology and RUT. When used to screen clarithromycin resistance genotypes, using probes targeting the 23S rRNA point mutations, the FISH test was more sensitive in detecting mixed susceptible and resistant populations than did the agar-dilution method. The authors emphasize that clarithromycin resistance should be assessed in biopsies both from the antrum and the corpus, as in one-third of children with mixed infection the resistant strains were found in the fundus only [52]. Woo et al. developed a dual-priming oligonucleotide (DPO)-based multiplex PCR to detect both H. pylori infection and the most common point mutations occurring in the 23S rRNA conferring resistance to clarithromycin (A2142G and A2143G), directly on gastric biopsy specimens. The DPO-based multiplex was slightly less sensitive in identifying H. pylori-positive cases than histology, but was able to identify more clarithromycin-resistant strains than the phenotypic methods. This assay proved to be fast, does not require expensive instrumentation, and can thus be valuable in countries with high prevalence of clarithromycin resistance [53]. Kawai et al. used fecal specimens to detect H. pylori and its resistance to clarithromycin using a nested PCR based on the 23S rRNA gene and sequencing of the amplicons, prior to H. pylori treatment. They obtained a 94.3% eradication rate in the tailored group and 71.4% in the control group [54].

Finally, Chisholm et al. assessed the potential benefits of the application to routine testing, of a novel algorithm comprising a panel of three previously described PCR assays for detection and antibiotic susceptibility testing of H. pylori. All culture-negative gastric biopsies were first tested for H. pylori and Helicobacter helimannii-like organisms by a multiplex PCR assay targeting vacA and 16S rRNA genes, respectively. Then, in the positive cases, antibiotic susceptibility to clarithromycin and tetracycline was assessed by real-time PCR probe hybridization and melting point analysis assays targeting the 23S rRNA and 16S rRNA, respectively. The authors demonstrated that PCR testing was particularly useful when H. pylori culture was unsuccessful, due to contamination of the biopsy or when the specimen transportation was delayed. Without this additional testing, 16.9% of all patients examined could have been misdiagnosed as H. pylori negative by culture only [55]. Finally, two articles were published using magnetic resonance spectroscopy (MRS). The first study concerned gastric biopsies obtained from patients with various diseases studied ex vivo by high resolution – magic angle
spinning – MRS (HR-MAS-MRS) and compared to ultrastructural data. Several amino acids, e.g. glycine, alanine, choline, and triglycerides, were identified as possible markers of differentiation toward neoplastic lesions. Such a technique could be applied in vivo [56]. In the second study, the metabolic profile of gerbils infected or not with *H. pylori* was studied on urine specimens. Results showed that *H. pylori* infection disturbs carbohydrate and amino acid metabolism and modifies the gut microbiota [57]. This method should open a new field of exploration of *H. pylori* infection.

**Conflicts of Interest**

To be confirmed.

**References**


Pathogenesis of *Helicobacter pylori* Infection

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**Keywords**
Host cell adherence, colonization, VacA, CagA, signaling, carcinogenesis, antimicrobial response.

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**Abstract**

*Helicobacter pylori* induces chronic inflammation of the gastric mucosa, but only a proportion of infected individuals develop peptic ulcer disease or gastric carcinoma. Reasons underlying these observations include differences in bacterial pathogenicity as well as in host susceptibility. Numerous studies published in the last year provided new insight into *H. pylori* virulence factors, their interaction with the host and consequences in pathogenesis. These include the role of bacterial genetic diversity in host colonization and persistence, outer membrane proteins and modulation of adhesin expression, new aspects of VacA functions, and CagA and its phosphorylation-dependent and -independent cellular effects. This article will also review the recent novel findings on the interactions of *H. pylori* with diverse host epithelial signaling pathways and events involved in the initiation of carcinogenesis, including genetic instability and dysregulation of DNA repair.

**Helicobacter pylori** Virulence Factors

**Helicobacter pylori** Genetic Diversity and Host Colonization

*Helicobacter pylori* is characterized by a high level of genetic diversity which can be important for the adaptation to the host stomach and for the clinical outcome of the infection. Differences in gene content among *H. pylori* isolates in Mexican patients with various gastric pathologies, including cancer, showed patterns of disease-associated genes [1].

The plasticity of the *H. pylori* genome derives from its natural competence for transformation by exogenous DNA, from recombination and from mutations. These properties are at the origin of an extensive allelic diversity occurring even in a single host. Insertion of chromosomal DNA fragments of 1300 bp length into the recipient chromosome, associated with active genetic recombination, was demonstrated [2]. During homologous recombination, Holliday junctions generated by the RecG and RuvAB helicases are resolved by RuvC. The RecG homolog of *H. pylori* is devoid of resolvase activity and provides an anti-repair pathway [3]. It was suggested that competition between repair and anti-repair pathways may provide a mechanism to generate strain diversity and to maximize fitness at the bacterial population level [3]. After natural transformation of *H. pylori*, the import of short DNA fragments (1294 to 3853 bp) interrupted by interspersed sequences of the recipient (ISR) (mean length of 82 bp) was shown to result in the formation of complex mosaic alleles [4]. The control of import length and initiation of the ISR formation was dependent on the DNA glycosylase MutY, a component of the base excision repair (BER) pathway.

In vivo, *H. pylori* is exposed to acidity and oxidative stress, causing bacterial DNA damage. AddAB have both nuclease and helicase activities similar to RecBCD. AddA (HP1553) confers resistance to oxidative stress-induced DNA damage. *addA* mutants display a significantly reduced DNA recombination frequency. In mice, AddAB and RecA are required for stomach colonization [5]. Their role in bacterial adherence is associated with gene conversion-like events, a mechanism selected in the host that abolishes BabA-dependent adherence [6]. These data are in support of DNA repair and recombination as essential *H. pylori* mechanisms to optimize bacterial adherence to mucosal epithelium and persistent colonization.

**Outer Membrane Proteins and Adherence**

The *H. pylori* genome contains about 30 *hop* gene paralogous encoding outer membrane proteins (OMP). Mutations in *hopQ* increased adherence of *H. pylori* to
AGS gastric epithelial cells, CagA translocation into host cells and cellular alterations, demonstrating the importance of HopQ for bacterial adherence [7]. The hopQ type I genotype was associated with higher atrophy scores than the type II genotype and was proposed as a marker for gastroduodenal diseases [8].

Expression of adhesins might be modulated by genetic changes. Phase variation via slipped-strand mispairing in repetitive nucleotide tracts modulate sabA expression. Goodwin et al. [9] reported that sabA alleles of multiple length in the polyT and CT repeat tracts near the sabAB 5′ end, are found in 25% of clones of strain 26695. This was also confirmed among multiple H. pylori isolates from a single patient [9]. This mechanism occurring during chromosomal replication suggests a selective pressure for SabA expression in the host, allowing bacteria to adapt but also to escape host immune response. In the same study, transcription of sabA was repressed by the acid-responsive ArsRS two-component signal transduction (TCST) system in vitro [9]. BabA and SabA bind to the Lewis B (Le^a) and to glycosphingolipids displaying a sialyl-dimeric Lewis X (sialyl-Le^a) respectively. In young mice with early acquisition of H. pylori infection, a higher sialyl-Le^a was correlated with persistence of stomach colonization [10]. A cag pathogenicity island (PAI)-dependent over-expression of a GlcNAc transferase (b3GnT5) was described in gastric carcinoma cell lines and associated with high H. pylori adherence [11].

Four binding modes for the H. pylori–mucin interaction are likely to play roles in various niches along the orogastric infection route and vary according to pH, gastritis status and bacterial strain [12]. Glycoprotein receptors within the human salivary proteome for the carbohydrate-binding H. pylori adhesins have been also identified [13]. Binding of H. pylori to salivary mucin MUC7 and agglutinin gp340 depended exclusively on SabA and BabA, respectively. Binding to MUC5B was mainly due to BabA. SabA was also found to bind the secretory component of the polymeric Ig receptor (SC) and Igz-chain (S-IgA-Hc). These interactions can modulate surface or adhesive properties of the bacteria along the digestive tract. In gastric precancerous lesions and in gastric carcinoma, the expression of MUC5AC and MUC6 is altered. Using the Rhesus macaque model, Cooke et al. investigated the effects on gastric mucins of experimental challenge with H. pylori during acute and chronic infection [14]. H. pylori induced gastritis with an acute and high transient decrease in diversity and low relative abundance of O-linked mucin oligosaccharides, suggesting that bacteria modulate gastric mucin glycoproteins during acute infection to promote colonization and persistent infection.

Trefoil factors are involved in repair of the gastrointestinal mucosa. MUC5AC is coexpressed with trefoil factor family (TFF)1, a member of small cysteine-rich proteins. Reeves et al. [15] showed binding of the core-oligosaccharide portion (rough form) of H. pylori lipo-polysaccharide (RF-LPS) to TFF1, at an optimum pH of 5–6. An increase in pH would cause inappropriate binding of bacteria close to the lumen, leading to removal by mucus turnover. In patients with active chronic gastritis, a reduced expression of TFF2 was observed, especially in those patients infected with CagA-positive strains. Authors propose that this reduction in expression could contribute to the damage induced to the gastric mucosa by H. pylori [16].

The Cytotoxin VacA

VacA is an important virulence factor in the pathogenesis of peptic ulceration and gastric cancer. This toxin can induce multiple cellular activities, including cell vacuolation, membrane channel formation, disruption of endosomal/lysosomal function, apoptosis, and immunomodulation. The mature monomeric form of VacA has 2 domains - p55 and p33 - important for its cellular activity. Ivie et al. [17] showed that the N-terminal of p55 is essential for VacA-induced vacuolation and for host cell membrane depolarization. This domain is important for the formation of VacA oligomeric structures, suggesting its role in the formation of anionic membrane channels. Further insight into the final steps of vacuole formation by VacA was provided by Mashima et al. [18], by demonstrating that the vesicle associated membrane protein 7 (VAMP7) is a partner of Q-SNARE syntaxin 7 in the process of lysosome–endosome fusion.

At the nucleotide level, the vacA intermediate (i)-region, which encodes part of the p33 VacA subunit, displays sequence variation. i1-type strains were associated with gastric carcinoma in an Iranian population [19], and were an independent predictor of peptic ulcer disease in an Italian population [20].

In addition to the previously described protein tyrosine phosphatase receptor (RPTP)-x and RPTP-β, Gupta et al. [21] identified sphingomyelin (SM) as a host cell receptor for VacA. SM was essential for VacA association with the cell membrane and for toxin-induced vacuolation. VacA binding to specialized membrane functional domains may have a biological meaning in cell signaling.

Tegtmeier et al. [22] showed that VacA can inhibit some CagA-induced responses on epithelial cells. VacA inhibited the activation of epidermal growth factor receptor (EGFR) and HER2/Neu, and subsequently
Erk1/2 MAP kinase, which are important for cell scattering and elongation. These results are also in agreement with previous findings suggesting that VacA and CagA downregulate each other’s effects on epithelial cells, potentially allowing \textit{H. pylori} interaction with cells whilst avoiding excessive cellular damage [23].

A novel mechanism underlying \textit{H. pylori}-induced inhibition of acid secretion by parietal cells was proposed by Wang et al. [24]. They showed that VacA interaction with parietal cells promotes calpain-mediated proteolysis of ezrin, disrupting the apical membrane-cytoskeletal interactions and inhibiting gastric acid secretion, mimicking the hypochlorhydric phenotype observed in \textit{H. pylori}-infected patients.

Tuo et al. [25] demonstrated that VacA inhibits prostaglandin E2-stimulated duodenal mucosal bicarbonate secretion by stimulating the release of mucosal histamine. These findings may have pathophysiologic relevance since the inhibitory effect of VacA on bicarbonate secretion may impair duodenal mucosal defense against acid injury, contributing to ulcer development.

The \textit{cag} Pathogenicity Island and CagA

The \textit{cag}PAI is a genomic region of 40 Kb containing about 30 genes encoding a type IV secretion system (T4SS). To gain more insight into the role of the T4SS on the outcome of gastric disease, Wiedemann et al. used the Mongolian gerbil model in a long-term infection experiment (2–64 weeks) [26]. Authors showed that the T4SS is essential for the induction of an early and severe corpus inflammation, associated with increased expression of proinflammatory cytokines and histopathologic changes such as atrophic gastritis and metaplasia. At late time points, only animals infected with T4SS-competent bacteria developed hypochlorhydria and hypergastrinemia in parallel to gastric ulcers and local dysplasia. Although gastric adenocarcinoma was not detected in any of the infected animals, they show that the Mongolian gerbil model parallels the multistep process of gastric carcinogenesis that occurs in humans.

The \textit{cag}PAI-encoded factor CagD was investigated and its crystal structure was determined [27]. In contradiction with previous findings [28], CagD was identified as an essential component of the T4SS that is required for CagA translocation into host epithelial cells, although not absolutely necessary for pilus assembly.

CagA, also encoded by the \textit{cag}PAI, is translocated into host epithelial cells by the T4SS. Lai et al. [29] showed that cholesterol-rich raft microdomains of AGS cells are crucial for efficient T4SS-mediated CagA translocation and phosphorylation, as well as for subsequent CagA-induced actin rearrangements and IL-8 secretion. These results suggest that \textit{H. pylori} is able to exploit host cellular cholesterol in ways additional to those for VacA intoxication and immune evasion.

\textbf{CagA EPIYA motifs and CagA Phosphorylation-Dependent Host Cell Effects}

After translocation into the host cells, CagA can be phosphorylated in tyrosine residues within Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs at the polymorphic C-terminus of the protein. Argent et al. showed by using \textit{H. pylori} strains displaying cagA microevolution [30,31], that strains with additional copies of the EPIYA-C motif significantly induce more IL-8 secretion in AGS cells after prolonged infection periods. Sequence data analysis also revealed that Western strains are more likely to undergo duplication of the EPIYA-C motif than East Asian strains undergo duplication of the D motif. Authors speculate that the highly active East Asian CagA with one D motif has no requirement to increase its virulence, whereas the less active Western CagA displays a dynamic capacity to increase its number of C motifs to become more virulent.

Basso et al. [20] showed that the magnitude of risk for gastric carcinoma and precursor lesions increases with increasing numbers of EPIYA-C motifs. In a multivariate model also including vacA genotypes and corpus \textit{H. pylori} colonization density, the number of EPIYA-C motifs was an independent predictor of risk for intestinal metaplasia, reinforcing that the characterization of the EPIYA region is important in defining disease risk.

During the initial stages of infection, CagA is phosphorylated by host Src kinases and later by Abl kinases. Phosphorylation-dependent effects of CagA include induction of actin cytoskeleton rearrangements in the host cell. Phosphorylated CagA has been reported to interact with the Src homology 2 (SH2) domains of Shp-2, Csk and Crk (reviewed in [32]). Further insight into how CagA can induce actin cytoskeleton rearrangements may arise from the work of Selbach et al. [33]. They have identified by quantitative proteomics PI3K, Shp-1, Ras-GAP1, and Grb7 as additional cellular interaction partners of CagA. Their results also indicate that an individual tyrosine phosphorylation site of CagA can interact with different cellular SH2 domains, suggesting that \textit{H. pylori} can manipulate multiple signaling pathways in parallel.

Botham et al. [34] used a transgenic \textit{Drosophila} model with inducible CagA expression to show CagA’s capacity to function as a receptor tyrosine kinase adaptor. They demonstrated that CagA can substitute for Gab
and restore developmental defects caused by the loss of the Drosophila Gab, including larval lethality and photoreceptor differentiation. Authors also provided evidence that CagA functions similarly to Gab since it required the Drosophila SHP-2 to exert its effect on photoreceptor development.

**CagA Phosphorylation-independent Host Cell Effects**

Unphosphorylated CagA can also elicit host cell responses such as disruption of tight and adherens junctions, loss of cell polarity, proinflammatory and mitogenic responses (reviewed in [32]). CagA binds to and inhibits PAR1b/MARK2 kinase activity, thereby disrupting junctional and epithelial cell polarity in epithelial cells (reviewed in [35]). CagA-PAR1b interaction is mediated by the CagA multimerization (CM) motif. Lu et al. [36] showed that the CM motif of East Asian strains binds PAR1b more strongly than that of Western CagA, and in Western strains the ability to bind PAR1b is proportional to the number of CM motifs. It was further demonstrated that the level of CagA-PAR1b binding influences the magnitude of junctional defects.

Another phosphorylation-independent cellular effect of CagA is activation of the STAT3 signaling pathway. Bronte-Tinkew et al. [37] showed in the Hep-2 cell line that *H. pylori* triggers tyrosine phosphorylation, nuclear translocation and STAT3 transcriptional activity in a CagA-dependent manner. In contrast to other bacterial pathogens that modulate STAT3 via autocrine activation by IL-6, *H. pylori*-mediated STAT3 activation occurs at the IL-6R level but is independent of the known activation ligands IL-6, IL-11, and LIF.

Bauer et al. [38] described a new mechanism by which *H. pylori* can increase the amount of signaling molecules on the surface of infected cells. Authors present evidence that upon prolonged infection, *H. pylori* increases EGFR surface expression by inhibition of receptor endocytosis and degradation. This occurs in a CagA-dependent but CagA phosphorylation-independent activation of c-Abl, which in turn phosphorylates a specific EGFR target site.

**Helicobacter pylori and Epithelial Cell Signaling Pathways**

The phosphatidylinositol 3-kinase/protein kinase B (PI3K)/Akt signaling pathway regulates diverse biological processes, including cell proliferation, survival, and migration. Several studies provided evidence that *H. pylori* activates the PI3K-Akt signaling pathway in epithelial cells [39–44]. In keeping with these observations, in the Mongolian gerbil model of infection hyperphosphorylated Akt was predominantly expressed in the gastric pit cells of *H. pylori*-infected animals [44]. However, discrepancies were found regarding the involvement of bacterial virulence factors in *H. pylori*-induced PI3K-Akt signaling. Differences in cell line models and in strains can strongly contribute to these discrepancies.

PI3K activity can be induced by receptor tyrosine kinases. EGFR activation [39–42], but not c-Met [41], was found to be involved in *H. pylori*-mediated PI3K-Akt signaling and was shown to be important in cell survival [39,42], migration [39], and IL-8 production [40].

It was also demonstrated in cell line models that glycogen synthase kinase 3β (GSK3), a downstream target of Akt, is phosphorylated and inactivated by *H. pylori* [40,41,43,44]. In unstimulated cells, GSK3β phosphorylates β-catenin, targeting it for ubiquitinylation and degradation. Sokolova et al. [41] showed that by suppressing GSK3β activity, *H. pylori* leads to inhibition of β-catenin phosphorylation and ubiquitin-dependent degradation, and to upregulation of T cell factor/lymphoid enhancer-binding factor (Tcf/Lef)-dependent transcription of cyclin D1. GSK3β suppression through PI3K-Akt activation by *H. pylori* may also be involved in NF-xB activation and IL-8 production [40,44].

**Early Events in *H. pylori*-induced Carcinogenesis**

Carcinogenesis results from accumulation of genetic changes and dysfunction of cellular mechanisms that normally maintain genome integrity. Machado et al. [45] showed a dysregulation of DNA repair with decrease of mismatch repair components during *H. pylori* infection, leading to accumulation of genetic instability in the gastric epithelium. These mechanisms include induction of a transient mutator phenotype in the nuclear genome, microsatellite instability (MSI), and mutations in mitochondrial DNA. In addition, disruption of the balance between cell proliferation and apoptosis during *H. pylori* infection may promote gastric carcinogenesis. Apurinic/apyrimidinic endonuclease-1 (APE-1) regulates the transcriptional activity of p53. Bhattacharyya et al. [46] reported an *H. pylori*-mediated acetylation of APE-1 that suppressed Bax expression and prevented p53-mediated apoptosis, with potential consequences for gastric carcinoma development. These studies highlight the role of *H. pylori* in the induction of genetic instabilities and impairment of DNA repair systems important for promoting the gastric carcinogenic process.
Antimicrobial Host Response

The Rhesus macaque model of *H. pylori* infection displays lesions similar to those found in humans. In this model, a cagA/PAT-dependent increase in mucosal inflammation, with an increased expression of antimicrobial molecules related to β-defensin-2 (BD2) and several additional innate host defense proteins that may be important for disease pathogenesis was reported [47]. Human β-defensin-4 (hBD-4) is frequently expressed in the gastric mucosa with the highest levels detected in cagA positive *H. pylori* gastritis [48]. Its induction is dependent on the activation of the p38 MAP kinase pathway. These studies demonstrated that *H. pylori* induces a differential expression of antimicrobial peptides, which are essential effectors of the innate immune response with functional relevance in host defense. These antimicrobial proteins may be less active against *H. pylori* than against other microorganisms, resulting in a modification of the gastric microbiota composition during host infection [47]. Dickson et al. characterized the gastric microbiota of patients with gastric carcinoma, and demonstrated that it was dominated by different microbial species with a relative low abundance of *H. pylori* [49]. Pathogenesis of *H. pylori* infection can be also modulated by lower bowel Helicobacters, as suggested by mice experiments showing an attenuation of the *H. pylori*-induced gastric proinflammatory lesions in a co-infection with *Helicobacter bilis* [50].

Conflicts of Interest

The authors have declared no conflicts of interest.

References

Inflammation, Immunity, and Vaccines for *Helicobacter pylori*

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**Abstract**

*Helicobacter pylori* infects almost half of the population worldwide and represents the major cause of gastroduodenal diseases, such as duodenal and gastric ulcer, gastric adenocarcinoma, autoimmune gastritis, and B-cell lymphoma of mucosa-associated lymphoid tissue. *Helicobacter pylori* induces the activation of a complex and fascinating cytokine and chemokine network in the gastric mucosa. Different bacterial and environmental factors, other concomitant infections, and host genetics may influence the balance between mucosal tolerance and inflammation in the course of *H. pylori* infection. An inverse association between *H. pylori* prevalence and the frequencies of asthma and allergies was demonstrated, and the neutrophil activating protein of *H. pylori* was shown to inhibit the allergic inflammation of bronchial asthma. During the last year, significant progress was made on the road to the first efficient vaccine for *H. pylori* that will represent a novel and very important bullet against both infection and gastric cancer.

*Helicobacter pylori* infects the stomach of more than 50% of the human population and represents the major cause of gastroduodenal pathologies, such as duodenal and gastric ulcer, gastric adenocarcinoma, autoimmune gastritis, and B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), and autoimmune gastritis. As in any infectious disease, the type of innate and specific immunity elicited is of crucial importance for protection, although an inappropriate response may contribute to the induction of immunopathology. This article will focus on the major findings on the host response and vaccines against *H. pylori* published over the past year.

**Natural Immunity**

*Helicobacter pylori* activates a wide spectrum of innate events resulting in a strong T helper (Th) 1 response. Mucosal natural defense against *H. pylori* infection depends on activation of both Toll-like receptors (TLR) and Nod-like receptors (NLR), which lead to the generation of Th1 response specific for *H. pylori*. Different components of *H. pylori* are able to activate innate immune cells. The neutrophil activating protein of *H. pylori* (HP-NAP) is a key factor in *H. pylori* TLR activation. HP-NAP induces interleukin (IL)-12 and IL-23 secretion in monocytes, dendritic cells, and neutrophils via activation of TLR2 [1]. *Helicobacter pylori* factors other than HP-NAP, such as the vacuolating cytotoxin A (VacA), the *cag* pathogenicity island (PAI), and the heat shock protein (HSP) 90 may contribute to the expression of IL-12p40 and to Th1 polarized response [2]. In particular *H. pylori* peptidoglycan, acting in concert with the bacterial type IV “syringe”, encoded by the *cag* PAI, plays an important role in activation of the cytoplasmic nucleotide-binding oligomerization domain (NOD) 1 in gastric epithelial cells and primes Th1 responses [3,4]. Another source of IL-12 might be natural killer cells, which can be elicited by *H. pylori* in infected patients [5]. Following *H. pylori* infection, gastric epithelial cells and monocytes produce other Th1-inducing cytokines such as IL-18. IL-18 levels in infected gastric mucosa correlated with the severity of gastric inflammation both in adults and children [6,7]. Different bacterial virulence factors, such as *cag* PAI and outer inflammatory protein (Oip) A, contribute to the induction of IL-18 production in gastric epithelial cells. In both gastric epithelial cells and monocytes/macrophages, *H. pylori* regulates the extracellular signal-regulated kinase/c-Jun-N-terminal kinase (JNK)-AP-1 pathway. Upregulation of IL-18 mRNA in monocytes is independent of *cag* PAI and OipA, whereas OipA and its related p38 pathway regulate IL-18 protein induction at...
the post-transcriptional level in a cag-independent way, and contribute to gastric injury [6]. *Helicobacter pylori*-infected patients with IL-18-607C/C and -137G/G genotypes have been shown to have higher IL-18 levels and severe gastric inflammation [8]. In a very elegant study in C57BL/6 mice, Kaparakis et al. demonstrated that transient elimination of macrophages during the early period of *H. pylori* SS1 infection reduced the gastric pathology, suggesting that macrophages contribute to the severity of gastric inflammation [9].

Neutrophil activation and mucosal IL-8 expression are associated with persistent infection. A new triggering receptor expressed on myeloid cells-1 (TREM-1) has been described on gastric epithelium and experimental data suggest that TREM-1 expression on gastric epithelial cells amplifies inflammation of the underlying gastric mucosa by upregulation of IL-8 [10]. A novel putative *H. pylori* outer membrane protein (HomB) associated with peptic ulcer disease has been extensively investigated. HomB induces the secretion of IL-8 by gastric epithelial cells, and *H. pylori* homB knockout mutant strains present reduced ability to bind to gastric epithelial cells and to induce IL-8 secretion, implying that HomB represents a novel virulence factor of *H. pylori* actively involved in *H. pylori*-induced inflammation [11]. Individuals with lower neutrophil oxidative burst activity might be more prone to *H. pylori* infection, due to reduced efficiency of neutrophil immune functions [12]. Moreover, in a different experimental setting, HP-NAP confirmed its ability to induce myeloperoxidase release from human neutrophils [13]. Furthermore, it has been shown that *Helicobacter pullorum*, an entero-hepatic *Helicobacter* species of avian origin detected in patients with acute diarrhea and inflammatory bowel disease, exerts a direct effect on human gastric (AGS) and intestinal (CaCo-2 and HT-29) cell lines, by inducing IL-8 production. The *H. pullorum*-induced IL-8 secretion requires bacterial adherence and lipo polysaccharides (LPS) and is mediated by nuclear factor (NF)-κB signaling, suggesting that *H. pullorum* might play a putative role in acute and chronic digestive diseases such as inflammatory bowel disease [14].

*Helicobacter pylori* LPS, another important bacterial factor that modulates the innate immune response, has been extensively studied for its pro-inflammatory activity. LPS has evolved differently in *H. pylori* communities through genetic modifications in fucosyltransferases that are involved in Lewis (Le) antigen expression. Some of the LPS variants facilitate adaptation and survival in the individual gastric mucosa [15]. Furthermore, Le antigen expression and fucosylation can have multiple biological effects, by affecting the development of innate and acquired responses that develop after infection, implying that the fucosylated secretor ABH antigens constitute a family of interactive members of the mucosal human innate system that tightly regulates host–bacterial interactions [16]. The host iron status may affect the nature of LPS expressed by *H. pylori*, by modifications in outer membrane vesicles [17]. Evidence suggests that long-term *H. pylori* infection can induce antibodies that cross-react with the gastric mucosa and that, in concert with H’K’-ATPase-specific autoreactive and cross-reactive T cells, contribute to the development of gastric autoimmunity and to mucosal atrophy [18,19]. The LPS may decrease *H. pylori* elimination from the gastric mucosa and promote infection persistence, by exerting an anti-phagocytic activity that is reduced by LPS-binding protein [20]. *Helicobacter pylori* proteins, such as VacA, may exert either inhibition or activation on different cell types. VacA exerts immune suppression by inhibition of antigen processing and presentation of antigen-presenting cells and by disruption of actin rearrangement and inhibition of calcium mobilization of T cells. VacA has different receptors on different cell types, such as CD18 on T cells [21], or sphyngomyelin on epithelial cells [22]. VacA can inhibit duodenal bicarbonate secretion via prostaglandin E(2) inhibition by a histamine-dependent mechanism in mice [23]. On the other side VacA induces IL-8 production in U937 cells via activation of p38 mitogen-activated protein kinase and intracellular Ca²⁺ release, leading to the activation of the transcript factors ATF-2, CREB, and NF-κB [24].

The mechanisms of inflammation induced by CagA have been further elucidated. Suzuki et al. demonstrated that nonphosphorylated CagA is able to promote inflammation by sequential activation of PI3 kinase/Akt signaling that, in turn, leads to β-catenin and NF-κB activation [25]. The CagA protein is also able to function both as a Grb2-associated binder protein (Gab) adaptor and to activate the Src-homology 2 domain containing tyrosine phosphatase (SHP-2) in a transgenic *Drosophila* model [26]. *Helicobacter pylori* induces gastric inflammation via a Cag-dependent mechanism both in Rhesus macaques and in gerbils [27,28]. On the other hand, Cag-independent signaling might lead to β1-integrin activation with JNK activation, which also promotes cell motility of gastric cancer cells [29]. Basu et al. demonstrated that the secreted peptidyl prolyl cis-, trans-isomerase HP0175 protein was able to bind to AGS cells by TLR4 and to transactivate EGFR and vascular endothelial growth factor production [30]. Using the A/JCr mouse model, *Helicobacter hepaticus* urease was shown to contribute to hepatic inflammation although it was not required for intestinal colonization [31].
Adaptive Immunity, Helicobacter, and Cytokine Network

T-helper cells orchestrate host defense against pathogens via different types of cytokine secretion and effector functions; however, an inappropriate response might lead to immunopathology. Current evidence suggests that in *H. pylori* infection, a predominant activation of Th1 cells with production of interferon (IFN-γ), IL-12, IL-18, and tumor necrosis factor (TNF-α) occurs in vivo in the gastric mucosa and contributes to tissue damage [32,33]. Accordingly, *Helicobacter* infection of lymphocyte-deficient mice fails to induce gastric inflammation. T-cell transfer into deficient animals then results in severe gastritis, suggesting that host T-cell responses to *H. pylori* play a key role in host damage [34,35].

The fine balance between protection and pathology in *H. pylori* infection is related not only to the major Th1 cytokines, such as IFN-γ, TNF-α, IL-12 [32], but also to IL-23 (a powerful Th1 and IL-17-promoting factor), IL-17 and IL-21 [1,36,37]. IL-17A mRNA and protein are associated with *H. pylori* lesional sites from human gastric biopsies [37,38]. When biopsies were cultured in vitro and IL-17 activity was blocked, there was a reduction in IL-8 gene expression implying that IL-17 might be a relevant factor driving IL-8 production and neutrophilic inflammation. In both humans and mice, the upregulation of IL-23 was found in gastric mucosa following *H. pylori* infection and was associated not only with IL-17 but also with the upregulation of many IFN signature transcripts [39]. IL-23 is present at high levels in the lesional tissue compared with the surrounding tissue. Blocking IL-23 activity resulted in a reduction of STAT3 and IL-17 expression [40]. Experimental evidence obtained in a mouse model of *H. hepaticus* infection suggests that the NF-κB subunit c-Rel modulates the expression of IL-23/IL-12 subunits and plays an important role in the development of innate and T-cell mediated inflammation [41].

The type of mucosal lymphocyte responses going on in the duodenum and in the stomach of *H. pylori*-infected patients were investigated in a highly endemic area of *H. pylori* infection, i.e. Bangladesh, and compared to Sweden. Comparable amounts of T and B cells were found in the stomach of Bangladeshi and Swedish infected patients, but there was a lower systemic antibody response in Bangladeshi patients. However, increased numbers of B cells and *H. pylori*-specific IgA antibodies were detected in the duodenum of Bangladeshi patients, suggesting a more intense inflammation going on in these patients frequently exposed to enteric infections [42]. In Swedish infected patients, increased gastric levels of the CCL28 chemokine and CCL28 mediated recruitment of gastric IgA-secreting cells were found, providing an explanation for the large influx of IgA-secreting cells to the gastric mucosa of *H. pylori*-infected individuals [43]. In a long-term follow-up of north-eastern European *H. pylori*-infected patients, Vorobjova et al. reported that different serological patterns were associated with histological manifestations of gastritis in the progression towards atrophic gastritis, in a long-term follow-up. Anti-CagA antibodies were a sign of gastritis activity and corpus atrophy, the prevalence of anti-canalicular antibodies significantly increased and paralleled the duration of *H. pylori* gastritis, whereas anti-HSP60 antibody levels indicated chronic inflammation of the antrum [44].

Interactions Between *H. pylori* and Asthma or Other Infections

Humans are colonized by a multitude of both beneficial and pathogenic microbial organisms, including *H. pylori*. Imbalances in the composition of bacterial microbiota are postulated to be a major factor in many human disorders [45]. Human and microbial cells continuously “cross-talk” to each other and influence their respective lives. Over the last century the incidence and severity of bronchial asthma have drastically increased in developed countries and it has been proposed that infectious agents can influence the development of allergic disorders, although the underlying reason has not been fully elucidated. Bronchial asthma and allergic diseases are sustained by Th2 inflammation and IL-4 production, which is strongly inhibited by IL-12 and IFN-γ, whereas *H. pylori* infection elicits a powerful Th1 response [46]. Interestingly, large epidemiological studies recently demonstrated a consistent negative association between *H. pylori* infection and the presence of allergic disorders, such as asthma and rhinitis, both in childhood and the adult urban population [47–50]. In allergic asthmatic patients, the typical Th2 response can be redirected in vitro toward Th1 by HP-NAP [1]. To address whether HP-NAP, a TLR2-ligand, could be beneficial in vivo for the prevention and treatment of bronchial asthma, it was administered via the intraperitoneal (systemic) or the intranasal (mucosal) route using a mouse model of allergic asthma. The in vivo (both mucosal and systemic) administration of HP-NAP prevents the classic allergic Th2 bronchial inflammation, by a strong inhibition of IL-4, IL-5 and via the increase of IL-12 production. However, no suppression of bronchial Th2 cytokines was observed in TLR2 knockout mice following HP-NAP treatment [46,51]. Altogether these results provide evidence that HP-NAP might be an important
part of the molecular and cellular mechanisms underlying the negative association between *H. pylori* infection and allergy.

In mouse *Trichinella spiralis* infection, another model of Th2-mediated disease, HP-NAP was also able to enhance an in vivo Th1 response and to exert a powerful anti-Th2 activity, targeting both the IL-5-induced eosinophilia and the IL-4-mediated hyper-IgE responses induced by parasitic infection [52]. Different microbial factors and other concomitant infections may influence the outcome of *H. pylori* infection. In a C57BL/6 mouse model of *H. pylori* infection, treatment with *Lactobacillus casei* and *Bifidobacterium lactis* resulted in a suppressive effect on *Helicobacter*-induced inflammation [53]. Accordingly, *Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus lactis* and bovine colostral preparation were able to reduce adherence and IL-8 production following *H. pylori* infection of AGS cells [54].

**Regulatory Cells**

CD25+Foxp3+ regulatory T cells (Treg) are a subset of T cells that are physiologically devoted to the maintenance of self tolerance. Treg play a crucial role in regulating the effector immune responses in the different districts of the organism by suppressing the activation and proliferation of antigen-specific T cells, and an abnormal Treg activation might lead to impaired tumor immunity. Treg are able to suppress T-cell responses, via both cell contact and by soluble factors, such as TGF (transforming growth factor)-β and IL-10.

*Helicobacter pylori*-induced gastritis is associated with a recruitment of Treg that correlates with the degree of bacterial colonization and mucosal TGF-β expression [55]. In a murine model CD4+ CD25+ Treg cells were able to induce anergy of CD25− T cells in response to *H. pylori* infection but were not required to maintain hyporesponsiveness [56].

Robinson et al. investigated the gastric Treg response of infected patients and demonstrated that in subjects with peptic ulcer disease, higher levels of IL-10-secreting Treg were present in the gastric mucosa, compared with those without ulcers. IL-10 inhibited IL-8 expression and activation of NF-κB induced by *H. pylori* in gastric epithelial cells, and enhanced *H. pylori* growth in a bacterial-cell co-culture model [57]. Accordingly, a significant increase of IL-10 serum levels was found in a subset of Turkish infected patients [58].

Furthermore, *H. hepaticus* infection of mice co-infected with diarrheagenic *Escherichia coli* resulted in an accumulation of Treg cells at mucosal level [59] resulting in exacerbated morbidity, with delayed recovery from weight loss and tissue damage. Thus, it can be speculated that Treg cells play a role in the lifelong persistence of *H. pylori* infection and that an inadequate regulatory response may contribute to the immunopathology of *H. pylori* infection.

**Gastric Cancer, Inflammation and Gastric MALT Lymphoma**

Low grade gastric MALT lymphoma represents the first described neoplasia susceptible to regression following antibiotic therapy eradicating *H. pylori* [60]. The neoplastic B-cell proliferation depends both on *H. pylori* stimulation and exhaustive T-cell helper activity in conjunction with defective T-cell killing [61,62]. Ferrand et al. showed that *H. pylori* strains can inhibit T-cell proliferation, favoring chronic persistence of the infection and anarchical B-cell proliferation predisposing the host to gastric MALT lymphoma [63]. Different factors may affect the onset and progression of gastric MALT lymphoma. The CXCR3 chemokine receptor is highly expressed on both activated T and B cells of gastric MALT lymphoma. Patients with CXCR3 expression showed a significantly increased risk of nonresponsiveness to *H. pylori* eradication therapy, regardless of sex, API2-MALT1 fusion or clinical stage [64]. The overexpression of B-cell-activating factor of the TNF family (BAFF) was associated with *H. pylori*-independent growth of gastric diffuse large B-cell lymphoma, implying that the BAFF autocrine signal transduction pathway may contribute to *H. pylori* independent growth of gastric MALT lymphoma [65]. Furthermore, the majority of MALT lymphomas were found to express class-switched immunoglobulins and to develop in an environment rich in Th2 cytokines [66].

*Helicobacter pylori* is a very important oncogenic factor for gastric adenocarcinoma and many studies have highlighted the role of chronic inflammation in the development of gastric cancer [67]. *Helicobacter pylori* may lead to gastric cancer via both a direct effect on epithelial cells and by the induction of different cytokines, especially IL-1β and IL-1 receptor antagonist (IL-1RN). Overexpression of even a single proinflammatory cytokine is sufficient to induce neoplasia by eliciting inflammation. The European EPIC-EUROGAST prospective study confirmed the association of IL-1RN polymorphisms with the risk of noncardia gastric cancer and indicated that IL-8-251T>A may modify the risk for gastric cancer [68]. These results were confirmed by many reports all over the world. A Turkish study reported that the bacterial risk factor *babA2* seemed to be an important predictor of gastric malignancies, and that the presence of the IL-1β-31TT genotype represented an important protective factor [69]. The
upregulation of IL-1β, IL-8, and cyclooxygenase (COX)-2 linked to gastric carcinogenesis was found in Brazilian patients [70]. In a Costa Rica dyspeptic population CagA status was found to be a risk factor for atrophic antral but not body gastritis whereas the pro-inflammatory cytokine polymorphisms IL-1β +3945 and IL-1RB were not associated with the atrophic lesions of dyspeptic populations [71]. In a Korean survey study, the genetic polymorphisms of IL-8, IL-6, and IL-10 were associated with the development of *H. pylori* induced gastroduodenal diseases [72]. TNF-α inducing protein has been shown to be a novel *H. pylori* factor able to induce TNF-α secretion, to enter the nucleus of gastric epithelial cells and to induce carcinogenesis in a *cag*-independent way [73]. During *H. pylori* exposure, the production of MIF and IL-8 by gastric epithelial cells leads to the expression and activation of epidermal growth factor receptor (EGFR) in a *cag* PAI – independent way [74]. The p53 protein family, including p73 protein, by acting in concert play an important role in the epithelial and inflammatory response to *H. pylori* related to gastric malignancy via induction of apoptosis and promoting alterations of cell differentiation [75].

Experimental evidence obtained by studying the eosinophil infiltration in gastric infected tissues of patients originated from areas with different rates of infection suggested that eosinophils and mast cells might have a dual role in *H. pylori* infection: they can downmodulate gastric inflammation and cancer development in low risk areas whereas they might promote inflammation and progression to malignancy of precancerous lesions in high risk areas [76].

**Vaccines**

Despite almost 20 years of efforts, no efficacious vaccine against *H. pylori* is currently available for humans. Immunization with different vaccine formulations, based on the use of selected antigens known to be involved in the pathogenesis of infection, such as those containing VacA, CagA, and HP-NAP or urease, have been shown to prevent experimental infections in animals [77]. BabA and SabA adhesins have also been proposed for an anti-*H. pylori* vaccine [78]. Although the efficacy of a vaccine against gastric *H. pylori* infection has been shown, little is known about the mechanisms of bacterial clearance. Not only a specific antibody response but a concerted action of cellular, molecular, and humoral responses are needed to give full protection against *H. pylori*. A vaccine based on *H. pylori* Sydney strain 1 lysate and cholera toxin (CT) adjuvant has been used to intranasally immunize mice and the protection achieved was high. The immunization resulted in a strong IFN-γ producing T-cell response associated with an increase in chemokines, such as MIP-2, KC and LIX, which attract neutrophils to the stomach and are important for *H. pylori* eradication [79]. An interesting study pointed out that dendritic cells play a critical role in supporting the effector cellular response needed for the development of a successful *H. pylori* vaccine [80].

Using a Th1 vaccine consisting of an *H. pylori* sonicate plus CpG oligonucleotides and a Th2 vaccine consisting of an LPS-depleted sonicate combined with CT, Taylor et al. demonstrated in a mouse model that, although the CpG sonicate vaccine induced stronger systemic and local immune responses, only the LPS-depleted sonicate CT toxin-conjugated vaccine resulted in effective protection [81]. A further mechanism of vaccine-induced protection is the effector response elicited by the leptin receptor signaling, that has been elegantly proposed by Wehzens et al. [82].

In two prospective, randomized, double-blind controlled studies, *Salmonella enterica* serovar Typhi Ty21a-based, oral live vaccines containing *H. pylori* urease or HP0231 protein were tested in *H. pylori*-negative volunteers. Both these vaccine preparations were well tolerated but did not confer satisfactory protection [83].

Over the last year, a very promising study entered the scene and will presumably lead to the launch of the first vaccine against *H. pylori* in humans. Malferttheiner et al. investigated the safety and immunogenicity of a vaccine consisting of recombinant VacA, CagA, and HP-NAP given intramuscularly with an aluminium hydroxide adjuvant to *H. pylori*-negative healthy subjects. This very important randomized single-blind Phase I study involved 57 *H. pylori*-negative volunteers and explored three different schedules (0, 1, 2 weeks; 0, 1, 2 months; and 0, 1, 4 months) and two dosages of each antigen (10 and 25 μg) versus alum controls. All of the subjects were followed for 5 months and 36 of them received a booster vaccination 18–24 months after the end of the first set of vaccination. In both vaccine and placebo recipients, only very mild adverse reactions were present on monthly schedule. All of the vaccinees mounted specific IgG and cellular responses to one or two antigens and 86% of vaccinated subjects responded to all three antigens. Both antibody and cellular memory responses could be elicited by vaccination between 18 and 24 months later. The safety and the immunogenicity results obtained in this study suggested that the intramuscular vaccine formulation consisting of VacA, CagA and HP-NAP plus aluminium hydroxide adjuvant represents a very promising candidate vaccine for the prevention of *H. pylori* infection [84].

Collectively these findings suggest that achieving a successful vaccine against *H. pylori* will have a great...
impact on global health because it will be beneficial not only for preventing \( H. \) pylori infection (a class I oncogenic factor) but also for the prevention of gastric cancer.

**Conflicts of Interest**

M.M.D.E. is inventor and applicant of patent EU05425666.4, WO2007039451 for potential use of HP-NAP as therapy of cancer, allergic, and infectious diseases.

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Helicobacter pylori and Non-malignant Diseases

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Abstract
It is well known that Helicobacter pylori infection is associated with many nonmalignant disorders such as gastritis, peptic ulcer, gastroesophageal reflux disease (GERD), gastric polyp, nonsteroidal anti-inflammatory drug (NSAID)/aspirin-induced gastric injury, and functional dyspepsia. In 2008, interesting articles on the association of H. pylori infection with these disorders were presented, some of which intended to reveal the mechanisms of inter-individual differences in response to H. pylori infection, and have demonstrated that genetic differences in host and bacterial factors as well as environmental factors account for these differences. A decline in the occurrence of peptic ulcer related to H. pylori was confirmed. An inverse relationship between H. pylori infection and GERD was also confirmed but the impact of gastric atrophy on the prevention of GERD remained debatable. For NSAID-induced gastric injury, eradication of H. pylori infection has been recommended. During this year, eradication of H. pylori infection was recommended for patients treated with antiplatelet therapy as well as aspirin and NSAID. It was also reported that for patients with functional dyspepsia, eradication of H. pylori offers a modest but significant benefit.

Helicobacter pylori-positive peptic ulcer (PU) is one of the most important indications for H. pylori eradication. However, it has been made clear that H. pylori infection is also associated with nonmalignant disorders other than peptic ulcer diseases (PUD) and that eradication of H. pylori is sometimes effective for the treatment of these disorders. There are inter-individual differences in response to H. pylori infection. One of the reasons for the inter-individual differences is genetics. Environmental factors are also associated with such differences. In 2008, several new polymorphisms associated with H. pylori-related disorders were reported. However, the main factors associated with the clinical outcome of H. pylori infection in each individual have not been fully elucidated. In this review, some interesting articles on the association of H. pylori infection with nonmalignant disorders published between April 2008 and March 2009 will be discussed.

Gastritis and H. pylori Infection

It is well known that H. pylori infection causes histologic gastritis. There are inter-individual differences in the severity or patterns of gastritis which are then associated with the further development of different kinds of disorders, such as duodenal ulcer (DU), gastric ulcer (GU), and gastric cancer. Genetic differences in host and bacterial factors have been considered to be one of the reasons for the inter-individual differences.

For the explanation of these inter-individual differences in response to H. pylori infection, polymorphisms of cytokines, such as interleukins (ILs) and tumor necrosis factor (TNF)-α, have been studied intensively since the year 2000. These cytokine polymorphisms are associated with different patterns of gastritis among different individuals. In 2008, several new polymorphisms associated with H. pylori-induced gastritis were reported (Table 1).

Tahara et al. studied the effects of Toll-like receptors (TLRs) on gastritis and found that TLR2-196 to -174ins alleles were associated with more severe intestinal metaplasia in patients older than 60 years and were correlated with severity of gastric mucosal atrophy and intestinal metaplasia in female subjects [1]. They also studied the polymorphisms of Regulated upon activation, normal T-cell expressed, and secreted (RANTES) and found that RANTES-28G carrier was associated with a reduced risk of developing more severe intestinal metaplasia in H. pylori-positive subjects aged 60 years and older and in female subjects [2]. Trejo-de la OA,
et al. also studied the influence of TLR polymorphism and observed that single-nucleotide polymorphisms (SNPs) in the TLR4 gene were associated with severe H. pylori-associated diseases and with a modified pattern of inflammatory cytokines and chemokines in the gastric mucosa infected with H. pylori [3].

H. pylori is also associated with lymphoid follicle gastritis, which is known to sometimes evolve into monoclonal mucosa-associated lymphoid tissue (MALT) lymphoma. Achyut et al. studied the association of TNF-α and IL-10 gene polymorphisms with gastritis and lymphoid follicle formation and found that IL-10-819T and TNF-α-308A alleles may increase the risk of gastritis and lymphoid follicle formation [4].

There have been several important reports on the polymorphism of bacterial factors. H. pylori strains have been classified into two groups: strains with high virulence and those with low virulence. The differences between the two groups are partly explained by the status of cagA and vacA, which are well known to be polymorphic. For vacA, strains with an s1/m1 genotype have been thought to be more virulent than those with s2/m2. However, Jafari et al. evaluated the effects of vacA genotypes on gastric inflammation and injury as well as clinical presentation in Iranian populations and found that the vacA genotypes and cagA status were not useful markers for gastroduodenal diseases in their country [5]. Chomvarin et al. from Thailand attempted to determine whether any correlation exists between genotypes of vacA, cagA, cagE, iceA, and babA2 and clinical manifestations in dyspeptic patients infected with H. pylori and concluded that neither a single gene nor a combination of vacA, cagA, cagE, iceA, and babA2 genes was significantly helpful in predicting the clinical outcome of H. pylori infection in their country [6]. However, Basso et al. studied cagA and vacA polymorphisms as well as the number of type C Glu-Profile-Tyr-Ala motif (EPIYA) (EPIYA-C) segments, which increase phosphorylation-dependent cagA activity in H. pylori-positive Italian patients with different disorders and they confirmed the association of cagA and vacA s1/m1 polymorphisms with PUD and cancers and noted that the most important factors in western countries were the number of cagA EPIYA-C segment for cancer risk and the intermediate region type of vacA for PUD risk [7]. Because the EPIYA-C segment is the Src homology 2 domain-containing protein tyrosine phosphatase (SHP-2)-binding site of cagA is clearly associated with RAS/Map kinase, EPIYA-C will be the key factor for elucidating the bacterial types and their corresponding clinical outcomes, including gastric cancer.

As stated before, a variety of polymorphisms from both bacterial and host sides were reported to be associated with the severity and/or the type of gastritis. In contrast, Kim et al. evaluated risk factors of atrophic gastritis and intestinal metaplasia with respect to H. pylori virulence factors (i.e., cagA, vacA m1, and oipA), and environmental factors (i.e., smoking and alcohol) and host polymorphisms (i.e., IL-1β-511, IL-1RN, TNF-A-308, IL-10-592, IL-10-819, IL-10-1082, IL-8-251, IL-6-572, GSTP1, p53 codon 72, and ALDH2) and found that the bacterial factors were important risk factors for atrophic gastritis but that environmental and host factors were more important for intestinal metaplasia [8]. The conclusion from this article is that to understand the inter-individual differences in response to H. pylori infection among different subjects, not only genetics of hosts and bacteria, but also environmental factors have to be studied. Therefore, the useful marker that predicts the individual response to H. pylori infection remains to be elucidated in relation to environmental factors.

### Table 1

Representative studies on genetics associated with pathogenesis of Helicobacter pylori-related gastritis published in 2008

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<th>Authors</th>
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<tr>
<td>Tahara et al.</td>
<td>TLR2-196 to -174ms alleles are associated with severity of gastritis and intestinal metaplasia.</td>
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<td>Tahara et al.</td>
<td>RANTES-28G allele decreases the risk of intestinal metaplasia.</td>
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<td>Trejo-de la et al.</td>
<td>TLR4 polymorphisms are associated with cytokine secretion.</td>
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<td>Achyut et al.</td>
<td>Tumor necrosis factor-alpha (-308G/A) and interleukin-10 (Δ19C/T) are associated with gastritis, especially follicular gastritis.</td>
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<td>Jafari et al.</td>
<td>Polymorphisms of cagA and vacA (S1/2, m1/2) are not associated with the clinical outcome of H. pylori infection.</td>
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<td>Chomvarin et al.</td>
<td>Polymorphisms of virulence factors (vacA, cagA, cagE, iceA, and babA2) are not associated with the clinical outcome of H. pylori infection.</td>
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<td>Basso et al.</td>
<td>cagA EPIYA-C segment is associated with the development of intestinal metaplasia.</td>
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<tr>
<td>Kim et al.</td>
<td>Environmental factors are important than the genetics of host and bacteria.</td>
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(NSAIDs)/aspirin, a major factor of PUD. Some interesting reports on PUD and *H. pylori* infection were published in 2008, mainly dealing with epidemiology and pathogenesis (Table 2).

Sung et al. performed a systematic review of the published literature concerning the prevalence and incidence of PUD [9]. The analyzed articles came from western countries. The main conclusion was that PUD remains a common condition despite decreasing incidence and prevalence owing to a decrease in *H. pylori* infection. In contrast, Wu et al. from Taiwan reported that a dramatic decrease in the incidence of admissions for complicated or uncomplicated PUD from 1997 to 2006 correlated with a significant increase in eradication therapy and use of proton-pump inhibitor (PPI) [10].

Eradication of *H. pylori* infection is known to be effective in the prevention of bleeding ulcers. van Leerdam et al. examined epidemiological surveys on gastrointestinal bleeding cases and observed that *H. pylori* infection is found in about 50% of bleeding PU patients [11]. Therefore, they concluded that all ulcer patients should be tested for *H. pylori* infection and eradication treatment should be given to those who are positive.

Cytokine polymorphisms have previously been shown to be able to modulate host response to *H. pylori* infection and to determine the occurrence of PUD. Vascular factors could play a role in the pathogenesis of PUD regardless of the main ulcerogenic agent involved, *H. pylori* or NSAIDs. Kim et al. evaluated whether the vascular endothelial growth factor (VEGF) polymorphism could predict susceptibility to PUD through modified angiogenic activities and found that the VEGF polymorphism -1780T/C could significantly predict the predisposition to PUD after exposure to etiologic risks [12]. To understand the importance of this polymorphism, previously reported SNPs, such as IL-1β and TNF-α, are needed for a comparative study.

Recently, endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR) were proposed for the treatment of early gastric cancer. The timing of *H. pylori* eradication on the process of ulcer healing after EMR or ESD has been controversial, because eradication of *H. pylori* restores gastric acid secretion, which sometimes induces the ulcers to bleed just after EMR or ESD. Cheon et al. prospectively evaluated the effect of *H. pylori* eradication on the healing of gastric ulcer after EMR and stated that *H. pylori* eradication might improve the ulcer’s healing rate after EMR [13]. Even if a supplementary examination is necessary, there seem to be cases in which eradication of *H. pylori* immediately after EMR is beneficial.

### Gastroesophageal Reflux Disease (GERD) and *H. pylori* Infection

Studies have shown that the prevalence of *H. pylori* infection is lower in GERD patients than in non-GERD subjects. Therefore, *H. pylori* infection has been considered to be possibly protective against the development of GERD. The fact that the eradication of *H. pylori* favors GERD and/or exacerbates symptoms in patients with GERD remains controversial. Several studies published in 2008/2009 dealt with this subject and again reported controversial results (Table 3).

Corley et al. performed a case-control study on a large population from California by matching patients with a new diagnosis of Barrett’s esophagus with patients with GERD and control subjects randomly selected from the base population [14]. The control group was original in comparison with the previous studies in which patients requiring gastroscopy for digestive symptoms other than GERD were included in the control group, with a possibility of overestimating the *H. pylori* rate. They found that *H. pylori* infection and cagA+ status were inversely associated with a new diagnosis of Barrett’s esophagus and that the association might be at least partly mediated through GERD. Somi et al. studied a more limited number of patients and found a similar inverse association between *H. pylori* infection and cagA status and reflux esophagitis [15]. However, Fass et al. studying the factors associated with refractory GERD found that the status of *H. pylori* infection played a very limited role in refractory GERD [16].

### Table 2 Representative studies on the association of Helicobacter pylori infection and gastroduodenal ulcers published in 2008

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<tr>
<td>Wu et al.</td>
<td>Increases in <em>H. pylori</em> eradication therapy and proton-pump inhibitor use decrease the incidence of gastric and duodenal ulcer diseases.</td>
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<td>Van Leerdam et al.</td>
<td>Around half of the bleeding ulcer patients are infected with <em>H. pylori</em>. Eradication therapy is recommended.</td>
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<tr>
<td>Kim et al.</td>
<td>VEGF-1780T/C polymorphism is associated with peptic ulcer risk.</td>
<td>[12]</td>
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<tr>
<td>Chen et al.</td>
<td>Eradication of <em>H. pylori</em> facilitates the ulcer after endoscopic mucosal resection.</td>
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Similarly, Grande et al. did not find any difference in the main characteristics of a group of 146 GERD patients according to their H. pylori status [17]. These findings suggest that H. pylori status plays no important role in the development of GERD and erosive esophagitis. Kim et al. compared risk factors for erosive esophagitis and nonerosive reflux disease (NERD) and found that the history of H. pylori eradication could be related to the risk of erosive gastritis, but not of NERD [18].

Several studies were performed to clarify the relationship between H. pylori status, gastric atrophy, and GERD. Anderson et al. performed a case-control study including a large number of patients with esophageal adenocarcinoma, Barrett’s esophagus, reflux esophagitis, and controls [19]. They found an inverse association of H. pylori seropositivity and also atrophy determined by the pepsinogen I/II ratio with esophageal adenocarcinoma, Barrett’s esophagus, and reflux esophagitis. However, although gastric atrophy was involved, it might not fully explain the inverse association with H. pylori infection. Similarly, Kwon et al., who compared a group of 45 patients having erosive esophagitis with a group of 66 control patients, found that the rate of infection of H. pylori was lower in the esophagitis group and the pepsinogen I/II ratio was higher than that in the control group, suggesting an inverse association between GERD and H. pylori-related gastric atrophy [20]. In contrast, Mon kemuller et al. did not find any correlation between serum gastrin and pepsinogen I and II with the severity of GERD [21].

Because continuous administration of PPI for GERD patients carries the risk of exacerbating the gastritis in the event of an H. pylori infection, the preventive eradication of H. pylori is recommended for patients with GERD who are in need of maintenance doses of a PPI, although eradication of H. pylori increases the risk of rendering the GERD ‘obstinate’. Wu et al. stated that, although H. pylori eradication may lead to more resilient GERD in a subset of patients, the benefits of H. pylori eradication outweighed the risks from the point of view of prevention of gastric cancer, especially in Asian populations with a high incidence of gastric cancer [22].

In conclusion, most of the articles published in 2008 confirm that H. pylori infection is inversely related to GERD, erosive esophagitis, Barrett’s esophagus, and esophageal carcinoma. Whether reduction of GERD is only mediated through H. pylori-induced corpus atrophy is still being debated. The concept that eradication of H. pylori is needed in GERD patients treated with PPI has not changed.

**Gastric Polyps and H. pylori Infection**

Several interesting papers on H. pylori infection and gastric polyps were published in 2008. Since some gastric polyps may disappear after eradication of H. pylori, the pathophysiologica role of H. pylori infection in the development of gastric hyperplastic polyps has been suggested. Ohtsuki et al. studied the pathophysiologic role of cagA using cagA transgenic mice and found that wild-type cagA transgenic mice developed gastric epithelial hyperplasia and some of the mice developed gastric polyposis and adenocarcinomas of the stomach and small intestine, suggesting that cagA is an oncogenic protein [23]. Interestingly, such pathologic abnormalities were not observed in transgenic mice expressing phosphorylation-resistant cagA, indicating the importance of cagA tyrosine phosphorylation in the development of H. pylori-associated neoplasms.
Cronkhite-Canada syndrome (CCS) is a rare syndrome characterized by multiple polyps of the digestive tract with symptoms that include loss of taste, hair loss, and nail growth problems. Chronic diarrhea and protein-losing enteropathy are often observed. The cause of the disease is unknown. Okamoto et al. prescribed H. pylori eradication therapy for an H. pylori-positive patient with CCS in Japan and noted a regression of polyps and resolution of clinical findings such as edema with the normalization of serum total protein and albumin levels [24]. This case report suggests the possible role of H. pylori infection in the pathogenesis of CCS. Whether this particular strain was cagA-positive is unclear, but most H. pylori strains in Japan are cagA-positive. Therefore, cagA status is assumed to be associated with the development of gastric polyps.

**NSAIDs/Aspirin-Induced Gastric Injury and H. pylori Infection**

No major original contribution to this subject was published in 2008. However, a consensus of the American College of Cardiology on antiplatelet therapy and NSAID use and the guidelines of the American College of Gastroenterology for prevention of NSAID-related ulcer complications have been recently published [25–28] (Table 4). Kiltz et al. reviewed the literature from a rheumatological point of view [29]. Similar recommendations were given. For antiplatelet therapy, the recommendation is to examine H. pylori infection in patients with a history of PUD and to eradicate H. pylori infection when present. However, PPI were recommended to prevent recurrence of complications. For NSAIDs, Kiltz et al. concluded from the review of the literature that it was well demonstrated that NSAID-naive users benefited from testing for H. pylori infection and subsequent H. pylori eradication therapy prior to the initiation of NSAID, but that H. pylori eradication alone did not offer protection from gastroduodenal injuries in chronic NSAID users [29]. To relieve patients with recent ulcer complications from further gastrointestinal (GI) events, eradication of H. pylori alone is insufficient and long-term acid inhibition is required. The management of H. pylori infection and the prevention of GI complications in NSAID users need to be examined case by case. Eradication of H. pylori is important in preventing gastroduodenal injury as a result of antiplatelet and/or NSAID therapy.

Epidemiologic studies have revealed that the incidence of gastric cancer is lower in subjects receiving NSAID and/or aspirin compared with nonusers of NSAID and/or aspirin. In 2008, a supportive study was reported. Li et al. studied the effects of aspirin on the development of heterotopic proliferative glands in H. pylori-infected Mongolian gerbils and found that aspirin alleviated H. pylori-induced hyperplasia and the development of heterotopic proliferative glands and also increased H. pylori-induced apoptosis [30]. They concluded that aspirin was responsible for the antineoplastic activities in H. pylori-related gastric carcinogenesis.

**Functional Dyspepsia (FD) and H. pylori Infection**

The role of H. pylori infection in FD has not been fully elucidated and the effect of H. pylori eradication is still controversial. In 2008, several reports on the effect of H. pylori infection on FD were presented (Table 5).

Selgrad et al. stated in a review that population-based studies have demonstrated that H. pylori is detected more frequently in dyspeptic patients than in controls and that H. pylori eradication therapy gives a modest but significant benefit in nonulcer dyspepsia cases and leads to long-term symptom improvement [31]. Consequently the “test and treat” strategy should remain the first option in patients with unexplored dyspepsia without alarm features in areas where H. pylori prevalence is greater than 20%. In western countries with a low prevalence of H. pylori, migrant communities may constitute a target group for the “test and treat” strategy [32]. An important study from Denmark evaluated the long-term effects of H. pylori screening and treatment of dyspepsia by determining the dyspepsia health-care consumption and quality of life in a large randomized community-based trial [33]. The prevalence of H. pylori in screened subjects was low (17.5%). The effect of eradication on the rate of dyspepsia was modest and was not statistically significant contrary to the

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<td>Bhatt et al.</td>
<td>Eradication of H. pylori is recommended in NSAID/aspirin users with a history of peptic ulcer.</td>
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<td>Kiltz et al.</td>
<td>Eradication of H. pylori is effective in patients who have never used NSAID/aspirin.</td>
<td>[29]</td>
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<tr>
<td>Li et al.</td>
<td>Aspirin inhibits the development of heterotopic proliferative gland in Mongolian gerbils infected with H. pylori.</td>
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consultation and sick leave rates which were significant; the incidence of ulcers also decreased significantly. A randomized placebo-controlled trial from Singapore showed that, in an Asian population with FD, eradication of H. pylori had a major beneficial effect when compared with a placebo, with a symptom resolution rate of 39% versus 3%, respectively, after 1 year [34]. Differences between eastern and western patients with FD could be related to the lower prevalence of reflux symptoms in the Asian patients.

de Artaza Varasa et al. studied whether antral gastritis, commonly associated with PUD, may predict a greater symptomatic response to H. pylori eradication in FD and found that there was a tendency of symptomatic benefit with H. pylori eradication in patients with antral gastritis [35]. Similarly, Koivisto et al. showed that DU patients (from 50 to 59 years of age) with antral neutrophil inflammation, were significantly predictive of symptomatic improvement after H. pylori eradication [36].

Tahara et al. attempted to clarify the association between 5HTR2A C102T polymorphism, CD14 gene C-159T polymorphism, and polymorphism in codon 158 of the COMT gene, and dyspeptic symptoms [37–39]. They found that neither 5HTR2A polymorphism nor CD14 gene C-159T polymorphism was likely to be associated with dyspeptic symptoms, but that the COMT genotype seemed to influence the susceptibility to dyspepsia. The role of genetics in the development of dyspepsia needs further evaluation.

The impact of H. pylori on symptom control in patients with long-term PPI treatment was studied by Raghunath et al. [40]. They found that H. pylori infection was associated with lower reflux symptom scores only in patients with GERD and uninvestigated dyspepsia.

**Conclusion**

H. pylori infection is associated with many nonmalignant disorders as described before. Genetics of hosts and bacteria as well as environmental factors are responsible for the inter-individual differences in response to H. pylori infection in different individuals. Unfortunately, the impact of newly discovered polymorphisms is still unclear. Therefore, comparative studies are needed to clarify the important SNPs associated with a response to H. pylori infection. Although the pathophysiologic role of H. pylori in nonmalignant diseases has not been fully elucidated, eradication of the bacteria is sometimes effective for the treatment of these disorders. Eradication of H. pylori infection has also been recommended for patients treated with NSAID/Aspirin and/or antiplatelet agents. Indeed, there are no disorders for which eradication of H. pylori infection is contraindicated; therefore, the “test and treat strategy” appears to be useful in H. pylori-positive patients with certain symptoms, such as dyspepsia. However, further studies are needed to clarify more precisely the association of H. pylori infection with these nonmalignant disorders, which will contribute to higher quality of clinical practice in the treatment of digestive diseases.

**Conflict of Interest**

None of the authors had any conflict of interest related to this manuscript.

**References**

4. Achyut BR, Tripathi P, Ghoshal UC, Moorchung N, Mittal B. Interleukin-10 (-819C/T) and tumor necrosis factor-alpha


Basic Aspects of Gastric Cancer

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Gastric cancer (GC) remains a considerable public health problem worldwide, and although its incidence and mortality rates have gradually decreased, GC is second only to lung cancer as the leading cause of cancer death [1]. The great majority of gastric malignancies are adenocarcinomas that can be divided into two histologic entities, intestinal and diffuse types, which exhibit distinct epidemiological and genetic patterns. Curative treatment of GC requires complete surgical removal of the neoplastic tissue, but even with curative intent the 5-year survival is only about 20–30%. The high mortality is mostly because of late diagnosis of the disease, creating an urgent need for new diagnostic markers and treatment modalities.

A widely accepted model for the development of GC is that the disease arises from *Helicobacter pylori* infection in a susceptible human host [2]. This gram-negative bacterium is acquired in childhood and persists in the stomach over decades. Case–control studies have shown that *H. pylori* seropositivity is associated with a significantly increased risk of GC (2.1–16.7 times greater than seronegative persons) [3–5], being considered a causative pathogen for gastric carcinogenesis. Inflammation may be the key promoting factor in the process of carcinogenesis induced by *H. pylori*. This bacterium possesses a unique array of features that makes it highly adapted to this ecological niche and persistent as a long-term infection of the gastric mucosa. The infection first induces chronic superficial (nonatrophic) gastritis, which can progress through chronic atrophic gastritis, intestinal metaplasia, and dysplasia toward GC. However, only a small number of infected patients will eventually develop GC (<1%) [6]. Besides family history, which is a risk factor independent of *H. pylori* infection, host genetic factors such as genes associated with inflammatory response probably participate in stomach carcinogenesis.

Genetic Susceptibility to GC

Susceptibility without evident familial clustering is, despite presenting the weakest genetic effects, the main category of inherited susceptibility to cancer. High penetrance of cancer-related genes accounts for a very low proportion of overall cancer incidence, while cancer-susceptible alleles, as a result of their high frequency, may account for a significant fraction of the overall cancer incidence. In recent years it has been shown that polymorphisms in several genes considered to be critical for gastric carcinogenesis, such as those involved in the inflammatory response to *H. pylori* infection [7–11], in the mucosal protection towards *H. pylori* infection [12–15], in the protection of DNA to oxidative damage [16], and in detoxification enhancement [17,18], may influence the risk of progression to GC.

The first published epidemiologic evidence indicating the association between an increased risk of GC and proinflammatory polymorphisms came from a study of the interleukin-1-beta (IL-1β) and IL-1 receptor antagonist genes (IL-1RN) [19]. Recently, the EPIC 2008 study
confirmed that proinflammatory IL-1RN genotypes are significantly associated with an increased risk of non-cardia adenocarcinoma in H. pylori-positive cases [7].

In addition to polymorphisms in interleukin genes, the polymorphisms in the promoter region of tumor necrosis factor-alpha (TNF-α) gene have been extensively studied in relation to GC. TNF-α is a pleiotropic cytokine mostly produced by activated monocytes and macrophages, which play a key role in the inflammatory response. Although several promoter polymorphisms have already been identified, most studies have focused on the TNF-α -308G>A single nucleotide polymorphism (SNP). Canedo et al. performed a case-control study for the TNF-α -308G>A polymorphism to determine the association with the risk of development of GC [8]. Their results indicate that the detected association between the proinflammatory TNF-308*A allele and the increased risk of GC is at least partially influenced by linkage disequilibrium (with an as yet unidentified locus) [8]. This study emphasizes the importance of extending single SNP association studies to haplotype-based approaches.

IL-16 is known for promoting the secretion of tumor-associated inflammatory cytokines by monocytes. Gao et al. [9] was the first to examine the association between SNPs of the IL-16 gene and GC. The authors demonstrated that the rs11556218 T/G polymorphism of the IL-16 gene was significantly associated with susceptibility to GC [9]. Interferon gamma (IFNγ) is one of the most important Th1-related cytokines which have been shown to promote gastritis. Individuals homozygous for the IFNGR1-56*C allele were shown to have a fourfold increased risk of developing early-onset GC when compared with those homozygous for the IFNGR1-56*T allele [10].

Carcinogens and toxins are metabolized via the xenobiotic pathway which is an important defense mechanism against carcinogenesis. Hence, polymorphisms on enzymes involved in the protection of oxidation and enhancement of detoxification of carcinogens may therefore participate in GC. Selenoprotein S (SEPS1) is a novel selenoprotein located in the endoplasmic reticulum (ER) and plasma membrane. It is involved in the control of the inflammatory response of ER. SEPS1 protects cells from oxidative damage and apoptosis, and is widely expressed in a variety of tissues. Recently, the -105G>A promoter polymorphism of SEPS1 was shown to be associated with levels of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α. It is known that the substitution of the allele A for G at position -105 reduces the promoter activity in HepG2 cells, and therefore also the IL-1β levels which are associated with GC. In a Japanese study comprising 574 individuals, it was demonstrated for the first time that the -105G>A polymorphism of the SEPS1 gene was associated with an increased risk of intestinal-type GC [17].

Glutathione-S-transferase (GST) is another important enzyme catalyzing conjugation of potentially mutagenic electrophilic compounds, with nucleophilic glutathione yielding less toxic and more water-soluble compounds, readily excreted via urine or bile. Both GSTT1 and GSTM1 genes of the GST super gene family exhibit either null or deletion polymorphism. Individuals homozygous for the null allele lack GST enzyme activity and thus have an increased risk for cancer. GSTP1 exhibits a polymorphism within its coding region (A to G transition at nucleotide +313), which leads to reduced enzyme activity. Tripathi et al. reported that the frequency of GSTT1*0 was higher in GC patients (diffuse-type) than in the controls. Analysis of combined GSTM1*0 and GSTP1*0 frequencies revealed that simultaneous deletion of both genes was associated with a 2–5 times higher risk of GC in comparison with the presence of both the genes [18].

### Intestinal-Type GC

Intestinal-type GC is believed to develop by a multistep process also known as Correa sequence, in which invasive cancer is preceded by atrophic gastritis and metaplastic and dysplastic lesions. Usually this sequence of events is initiated and promoted by persistent H. pylori infection. However, only a fraction of infected persons develop cancer. Therefore, knowledge of cancer risk in relation to premalignant lesions in the stomach is an important basis for making decisions on surveillance and treatment of these patients. Previously reported progression rates to GC vary considerably and even for dysplasia the range is as high as from 0% to over 70% per year. Understandably, surveillance strategies of patients with such lesions are highly controversial. De Vries et al. analyzed the GC risk and surveillance practice among Dutch patients (n = 92,250) who were diagnosed with a premalignant lesion in the stomach between 1991 and 2004 using the Dutch Nationwide Histopathology Registry [20]. Follow-up data were collected and evaluated until December 2005, and they show that the distribution of histologic findings were 67% for intestinal metaplasia, 24% for atrophic gastritis, 8% for mild-to-moderate dysplasia, and 0.6% for severe dysplasia. The annual incidence of GC was 0.1% for atrophic gastritis, 0.25% for intestinal metaplasia, 0.6% for mild-to-moderate dysplasia, and 6% for severe dysplasia within 5 years after initial diagnosis. Importantly, only 61% of the patients with severe dysplasia
and 26–38% for the other lesions were re-evaluated by endoscopy. These figures seem alarmingly low, considering that the relative risk for GC in this study was estimated to be about 40-fold for severe dysplasia when compared with patients having atrophic gastritis. It is also noted that patients with mild-to-moderate gastric dysplasia have a similar or even higher risk of cancer than patients with Barrett’s esophagus. One of the major conclusions is that endoscopic surveillance at short intervals is warranted in patients with gastric dysplasia.

Knowledge of individual risk of progression of premalignant gastric lesions to GC would be useful information in planning surveillance strategies, especially for low-risk lesions such as atrophic gastritis and intestinal metaplasia. In addition to environmental factors, distribution and extent of histologic lesions in the stomach, and H. pylori virulence factors, host genetics play a role in gastric carcinogenesis. One of the most consistent and strongest association with GC and polymorphisms is the IL-1β gene, as recently reviewed by McNamara and El-Omar [21]). IL-1β is a proinflammatory cytokine that also inhibits acid secretion in the stomach, and certain polymorphisms in this gene lead to an increased risk of noncardia GC in the presence of H. pylori infection. Tu et al. have now published a transgenic mouse model that enlightens the role and possible mechanisms of the procarcinogenic effects of IL-1β [22]. In this paper, it is shown that stomach-specific expression of IL-1β leads to gastric inflammation and eventually to neoplastic changes, including intramucosal adenocarcinoma, that were more severe when mice were infected with Helicobacter felis. Furthermore, in this mouse model myeloid-derived suppressor cells seemed to contribute to the carcinogenic cascade while T and B cells were not needed for this phenomenon. Interestingly, IL-1β-activated Nuclear factor-kappaB (NF-kB) in these cells led to an increase in IL-6 and TNF-α production. It has been hypothesized that myeloid-derived suppressor cells could contribute to immunoresponse, angiogenesis, and tumor invasion. However, since these cells were not specifically deleted in this study, a role of other cells (such as neutrophils, macrophages, dendritic cells, myofibroblasts and endothelial cells) cannot be excluded. This study thus shows that IL-1β is sufficient in promoting inflammation and carcinogenesis in the stomach.

Tumor suppressor gene p53 is a marker of poor prognosis in many malignant diseases, and inactivating mutations of the p53 gene can be found in 38–71% of GC [reviewed in Ref. 23]. In addition, p53 mutations have already been found in intestinal metaplasia and in dysplastic lesions, suggesting that the gene’s inactivation may be an early event in gastric carcinogenesis. Indeed, Szoke et al. published that the RR genotype of codon 72 was found to be associated with a reduced incidence of intestinal metaplasia among H. pylori-infected patients [24]. The p53 gene contains several polymorphic sites of which the polymorphism of codon 72 has been most extensively studied in GC. However, the results are conflicting, and a recent meta-analysis concluded that the p53 codon 72 polymorphism may be associated with GC but only in Asian cohorts [25]. One possible explanation for these conflicting results is owing to the fact that the p53 family also includes other proteins, namely p63 and p73. Both of these proteins share structural similarity with p53, activate p53 target genes and are involved in regulation of apoptosis. The scenario is somewhat complex, since certain subforms of p63 and p73 proteins can act in a dominant-negative manner towards p53. Wei et al. recently demonstrated that H. pylori infection of gastric epithelial cells in vitro and in vivo in mice lead to upregulation of p73 protein [26]. These results suggest that p73 may play an important role in the pathogenesis associated with H. pylori infection, and that alterations in p73 gene may play a role in gastric carcinogenesis.

Diffuse-Type GC

Intestinal-type GC predominates in high-risk geographic areas and shows a correlation with the prevalence of H. pylori infection. Diffuse-type GC, in contrast, is more uniformly distributed and is apparently unrelated to H. pylori prevalence. Owing to its development underneath the gastric mucosal surface, diffuse GC is usually diagnosed at an advanced stage and is consequently associated with a poorer outcome. It can be further subdivided into poorly differentiated carcinoma, and signet-ring cell carcinoma (SRC). It is suggested that the latter is an initial, differentiated form of diffuse GC that may evolve into poorly differentiated carcinoma [27]. Moreover, and despite the decreasing incidence of GC worldwide, the incidence of diffuse GC in the form of SRC is increasing. At the molecular level, diffuse-type GC can be distinguished from intestinal-type GC on the basis of the cell–cell adhesion molecule, E-cadherin. This molecule is the key component of the epithelial adherens junction and as such is required for functional intercellular adhesion within epithelial sheets. E-cadherin is downregulated very early during diffuse GC development, suggesting a role in the initiation of this disease. In fact, a causal relationship between E-cadherin deficiency and the initiation of diffuse GC has been established. In a N-methyl-N-nitrosourea-treated mice model homizygous for the
E-cadherin gene (cdh1+/−), the presence of a second CDH1 hit is the earliest observable stage of human diffuse GC, also providing evidence for epigenetic downregulation of E-cadherin as an initiator of malignancy [28].

An alternative mechanism to explain loss of function of E-cadherin in diffuse-type gastric transformation may involve the epithelial–mesenchymal transition (EMT) regulator TWIST through crosstalk with Hedgehog (Hh) signaling. The Hh signal activation selectively occurs in diffuse-type GC and blocking of Hh signaling inhibits the growth of GC cells [29]. Transforming growth factor-β (TGF-β), which is a multifunctional cytokine, is a potent inhibitor of epithelial cell proliferation. Moreover, TGF-β may promote tumor growth by inducing the epithelial cells to undergo EMT. Inhibition of TGF-β signaling has also been reported to prevent progression and metastasis onset in diffuse GC, mainly because of its ability to enhance angiogenesis [30].

**Role of Stem Cells in GC**

In the last years several lines of evidence have suggested that stem cells play an important role in gastric carcinogenesis. Houghton et al. showed that *H. pylori*-induced inflammation can cause migration of bone marrow-derived stem cells to the gastric mucosa, where they may subsequently transform into GC lineages [31]. McDonald et al. demonstrated that mitochondrial DNA (mtDNA) mutations establish themselves in stem cells within normal human gastric body units, and are passed on to all their differentiated progeny [32]. Mutated units can divide by fission to form patches, with each unit sharing an identical, mutant mtDNA genotype. These data show that human gastric body units are clonal, contain multiple multipotential stem cells, and provide definitive evidence for how mutations spread within the human stomach, and show how field carcinization develops [32].

Apart from its role in embryonic development and tissue regeneration, Hh signaling is involved in adult stem cell maintenance. Hh signaling is activated in GC tumors, and appears to be crucial for the differentiation of gastric progenitor cells into mucus and parietal cells [33]. The proposed model is that cancer develops from tissue progenitor cells after chronic stimulation from various injuries (e.g., *H. pylori*-persistent infection), and that during the repair process, developmental signaling pathways activate tissue progenitor cells. Chronic stimulation may ultimately result in irreversible activation of the signaling pathways which leads to cancer formation. Mesenchymal stem cells (MSCs), a subtype of stem cells with great capacity of self-renewal and differentiation, have been isolated from several tumors. The question of whether a group of MSCs exists in GC has arisen and, for the first time, MSCs have been isolated from tumors of GC patients [34]. Altogether, the expanding field of gastric cancer stem cell biology may offer novel avenues of research with impacts on the diagnosis and treatment of cancer.

**Conflicts of Interest**

The authors have declared no conflicts of interest.

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Early diagnosis and efficient therapy for gastric cancer (GC) remain an ongoing challenge for health systems worldwide [1]. Approximately 80% of patients are diagnosed in an advanced stage with no curative treatment options. Surgical treatment with curative intent has a general 5-year survival rate of approximately 24% [2]. Currently, the major task is to define a general applicable stage-adjusted algorithm that not only respects the outcome of each treatment modality concerning mortality and morbidity, but also the post-interventional quality of life.

Development of adequate regimes for adjuvant as well as neoadjuvant or peri-operative systemic chemotherapy will have a significant role in the future [3,4]. For palliative chemotherapy taxan- and platinum-based combination-therapies are the standard of care. Modified regimens are under evaluation to lower treatment-related toxicities and to improve the quality of life [5–10].

This review gives a brief overview of articles published between March 2008 and April 2009 on the achievements in prevention, diagnosis and management of GC with an emphasis on *H. pylori* eradication as a means for successful prevention.

**Primary Staging**

Park et al. evaluated the diagnostic potential of EUS compared with multidetector-CT for restaging of patients after neoadjuvant systemic therapy (Docetaxel in combination with Cisplatin) in 40 Korean patients [11]. Both modalities revealed comparable results, but in a multivariate analysis EUS-documented downstaging was an independent prognostic factor for overall survival. In conclusion, EUS should be standard for evaluation of local tumor response after neoadjuvant treatment [11].

Nitti et al. evaluated the prognostic value of subclassification of T2-stage disease into T2a (muscularis propria) and T2b (subserosa) and related treatment algorithms [12]. In a retrospective analysis of 373 patients treated for GC with curative intention, the tumor-related mortality risk for the T2a stage was comparable to the T1 stage whereas for the T2b stage it was...
similar to the T3 stage disease. Thus, subclassification of T2-tumors is recommended for planning the therapeutic strategy [12].

**Curative Gastric Resection**

Laparoscopic resection has been shown to be a safe and adequate curative treatment in early gastric cancer (EGC), including stage I and II tumors [13]. This approach results in favorable outcome not only concerning treatment-related aspects (e.g. intraoperative blood loss, total amount of anetics used, post-operative hospitalization period) but also concerning physical, emotional, social and general symptom scales compared to open gastrectomy [14].

In a Korean study open partial gastrectomy was compared with total gastrectomy. Although overall survival was comparable in both groups (99.2% vs 98.5%, respectively, at final follow-up), post-operative complications occurred significantly more often in patients that had only proximal gastrectomy (61.8% vs 12.6%) [15]. Therefore, total gastrectomy and Roux-en-Y anatomical reconstruction is superior to limited proximal resection concerning post-operative complications.

There is still ongoing debate about the required extent of lymphadenectomy for adequate curative treatment of localized GC. In Asian countries, D2 lymph node dissection represents the standard of care whereas in several Western countries D1 dissection is still performed.

Sasako et al. evaluated the efficacy and safety profile of additional dissection of the paraaortic lymph nodes (PAND) in comparison to regular D2 lymphadenectomy in patients with preoperative tumor stage of T2b, T3 or T4 [16]. Major surgical complications (e.g. anastomotic leakage, pancreatic fistula, abdominal abscess) were not statistically different between the groups. There was no difference in 5-year overall survival between patients with D2 or D2 + PAND dissection (69.2% vs 70.3%, respectively; HR 1.03; 95% CI 0.77–1.37). In conclusion, more extended lymphnode dissection than D2 cannot be recommended for standard GC treatment [16].

**Endoscopic Versus Surgical Treatment**

Endoscopic treatment of EGC is an established method for a curative attempt because of a significant improvement in quality of life in comparison to more radical surgical techniques. Standard procedure represents the endoscopic mucosal resection (EMR) for elevated EGC <2 cm in diameter and small (<1 cm) depressed tumors without ulceration according to the Japanese guidelines [17]. Generally this approach was only legitimate for mucosal defined tumors, whereas by the recently introduced technique of endoscopic submucosal dissection (ESD), tumors involving the upper submucosal layers can also be treated using an insulation-tipped diathermy knife (alternatively a hook knife). A further advantage of this method is the “en bloc” resection of the specimen that allows a more precise pathological assessment concerning gross and microscopically complete tumor resection.

In a feasibility study in Japan, 551 consecutive patients with 589 EGCs were treated with ESD and received a median follow-up of 30 months (6–89) [18]. Inclusion criteria were (1) mucosal cancer with ulcer findings and largest diameter ≤3 cm, and (2) minute submucosal invasive cancer with largest diameter ≤3 cm (<500 μm distance from the muscularis mucosa). Curative resection was achieved, when vertical and lateral tumor margins were free of malignant tissue, no submucosal invasion deeper than 500 μm from the muscularis mucosa was detected, and lymphatic or vascular involvement was absent.

“En bloc” resection was achieved in 94.4% with 94.7% defined as curative resection. There were no treatment related deaths; minor complications were bleeding (1.8%) or perforation (4.5%), both being endoscopically manageable in all cases. During follow-up, local recurrence was documented in three cases only with non-curative resection and in one case after piece-meal resection (all of these cases underwent gastrectomy with D2 lymph node dissection). Metachronous GC occurred in 13 patients after curative resection, and in one after non-curative resection within 12–42 months. The 3-year and 5-year survival rates were 98.4 and 97.1%, respectively, with death occurring due to other tumors or heart disease [18]. Long-term follow-up data are needed for an adequate comparison with related outcome of surgical procedures.

Rescue treatment after non-curative resection should be surgical. However, for well-selected patients re-endoscopic treatment could be considered [19,20].

**Helicobacter pylori Eradication in the Prevention of Recurrence**

The efficacy of primary prevention of GC by eradication of H. pylori has been confirmed in several studies [21]. An important issue was that once preneoplastic changes (gastric atrophy and intestinal metaplasia) are established, prevention of further progression to invasive cancer is more unlikely to occur [22]. The so-called “point of no return” has been identified to be critical.
for an effective prevention of GC incidence or recurrence. Watari et al. examined the effect of *H. pylori* eradication on the histology and cellular phenotype of gastric intestinal metaplasia. They showed that *H. pylori* eradication changes the cellular phenotype of gastric intestinal metaplasia, which might be an important factor in the reduction of gastric cancer incidence after successful eradication [23].

Unexpectedly and with more question marks on the “point of no return” theory, Fukase et al. demonstrated that even after endoscopic resection of early GC, recurrence of metachronous GC is significantly reduced by *H. pylori* eradication [24].

In a multicenter, open-label randomized trial, 544 patients who underwent EMR of EGC received either eradication treatment against *H. pylori* (lansoprazole 30 mg, amoxicillin 750 mg and clarithromycin 200 mg, each twice daily) or a placebo regimen. Follow-up endoscopy was performed at 6, 12, 24 and 36 months. Primary endpoint of the intention-to-treat analysis was occurrence of GC at a site other than the primary treated site of the stomach (metachronous GC). At the 3-year-follow-up, metachronous GC developed in 9 of 272 patients who received eradication treatment (3.3%) and in 24 of the 272 placebo patients (8.8%) resulting in an odds ratio (OR) of 0.353 (95% CI 0.161–0.775, \(p = 0.009\)). In the modified intention-to-treat analysis adjusted for loss to follow-up and respecting the patient population that had received at least one post-treatment assessment of tumor status, an HR for metachronous GC of 0.339 was documented (95% CI 0.157–0.729; \(p = 0.003\)). From these findings, it can be stated that eradication of *H. pylori* is an effective method for prevention of metachronous GC after endoscopic treatment of EGC and should be routinely applied. These data confirm previous observations which have been published by other authors [25,26].

A further beneficial effect of post-interventional eradication of *H. pylori* was demonstrated by Cheon et al. [27]. Of 47 patients who had undergone EMR for EGC, in 21 *H. pylori* infection was cured whereas 26 patients were either treated with a proton-pump inhibitor (PPI) alone or the eradication therapy failed. At 4 weeks post-treatment, there was a significant difference concerning healing of the treatment-induced ulcers, with the group with successful *H. pylori* eradication showing superior ulcer reduction rates [27].

Precancerous Lesions

For adequate individualized risk assessment in planning prevention strategies, the question of whether gastric atrophy and intestinal metaplasia (IM) are premalignant rather than paramalignant lesions is still under debate. Most data point to an increased risk of gastric carcinogenesis if glandular atrophy, IM and even more so if dysplastic changes are detected [28].

In a large retrospective analysis, de Vries et al. evaluated data of 92,250 patients who were filed in the Dutch Nationwide Histopathology Registry for the period from 1991 until 2004. Follow-up of the registered patients was analyzed until 2005 [29]. Among these patients 22,365 (24%) were diagnosed with atrophic gastritis, 61,707 (67%) with IM, 7616 (8%) with mild/moderate dysplasia and 562 (0.6%) with severe dysplasia. Endoscopic and histopathologic follow-up was performed in 26% of patients with atrophic gastritis, 28% with IM, and 38% with mild/moderate dysplasia compared to 61% with severe dysplasia (\(p < 0.001\)). In the follow-up group, the annual incidence of GC increased according to the severity of the mucosal alteration present in 0.1% for patients with atrophic gastritis, 0.25% with IM, 0.6% with mild/moderate dysplasia and 6% with severe dysplasia. The resulting HR for severe dysplasia was 40.14 (95% CI 32.2–50.1). Further independent risk factors in the multivariate analysis were male gender (HR 1.5; 95% CI 1.3–1.7) and age (HR for 75–84 years 3.75; 95% CI 2.8–5.1) [29].

The cancer risk in patients with mild/moderate dysplasia was comparable to the risk for the development of colorectal cancer after removal of colonic adenomas. However, no recommendation for surveillance has been proposed. So far, the best estimate for the regression in histopathology scores can be calculated as a function of the square of the time the patient is *H. pylori* negative after eradication therapy [30]. There is an important need for guidelines to determine at what intervals patients will require endoscopic control.

Population-based Screening

Population-based screening and treatment of *H. pylori* infection most likely represents the current best option for primary prevention of GC, but several aspects need to be considered, such as timing, methods and cost-effectiveness in various regions of the world. Certain populations, such as those in East Asia, have a high incidence of GC compared to populations in Africa, South Asia or Europe and this is probably linked with certain *H. pylori* strains. In high risk populations, mainly *H. pylori* with East Asian type CagA are present [31].

In a recent calculation for a high risk region in China, an empirically calibrated model of GC was used to estimate the reduction of lifetime cancer risk, life-expectancy and screening, as well as treatment-related costs [32]. Three options were considered: (i) single...
lifetime screening at age 20, 30 or 40; (ii) single lifetime screening followed by rescreening individuals with negative results, and (iii) universal treatment for *H. pylori* infection at age 20, 30 or 40.

Screening and treatment in individuals at the age of 20 resulted in adequate reduction of the lifetime risk for GC (males: 14.5%; females 26.6%) with costs below US$1500 per life year saved. By application of universal treatment, the risk reduction was even increased by 1.5% and 2.3%, respectively, but the incremental cost-effectiveness rates exceeded US$2500 per life/year saved. Assessing persons at an older age or rescreening of negative individuals was not cost-effective. Results of prospective trials on a global scale are needed to support these theoretical estimations.

**Conclusion**

The development of EMR/ESD enables a better curative access with preservation of a good quality of life in EGC. Palliative therapies for GC have still not achieved an important breakthrough. In the development of cost-effective primary prevention strategies the detection and treatment of *H. pylori* infection is the best available option [33]. Treatment of the infection is never too late as it also has the potential to prevent recurrence of GC as well as development of metachronous GC after endoscopic resection in some patients.

**Conflict of Interest**

P. Malfertheiner is involved in advisory boards and lectures with Astra Zeneca, Nycomed, Abbott and Novartis. All other authors have declared no conflicts of interest.

**References**


Treatment of *Helicobacter pylori* Infection

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Abstract

This article aims to examine current best practice in the field reference to first-line, second-line, rescue and emerging treatment regimens for *Helicobacter pylori* eradication. The recommended first-line treatment in published guidelines in Europe and North America is proton pump inhibitor combined with amoxicillin and clarithromycin being the favoured regimen. Rates of eradication with this regimen however are falling alarmingly due to a combination of antibiotic resistance and poor compliance with therapy. Bismuth based quadruple therapies and levofloxacin based regimes have been shown to be effective second line regimens. Third-line options include regimes based on rifabutin or furazolidone, but susceptibility testing is the most rational option here, but is currently not used widely enough. Sequential therapy is promising but needs further study and validation outside of Italy. Although the success of first line treatments is falling, if compliance is good and a clear treatment paradigm adhered to, almost universal eradication rates can still be achieved. If compliance is not achievable, the problem of antibiotic resistance will continue to beset any combination of drugs used for *H. pylori* eradication.

The treatment of *Helicobacter pylori* infection has posed conundrums for clinicians since the bacterium was first discovered in the early 1980s. The challenges go beyond finding the correct combination of antibiotics and manipulation of gastric pH to ensure eradication and include avoiding the development of antimicrobial resistance and ensuring compliance with prescribed treatment. The Maastricht-III consensus stated that for an eradication treatment regime to be considered effective, it would need to achieve an intention-to-treat eradication rate in excess of 80% [1]. However, in recent times, eradication rates in practice for many of the most common regimens have fallen well below these levels, generally due to the interwoven factors of poor compliance with medication and antibiotic resistance [2].

**First-Line Therapy (Standard Triple Therapy)**

The combination of a proton pump inhibitor (PPI) with two antibiotics has been accepted as the first-line therapy of choice for *H. pylori* eradication since a randomized control trial in 1996 [3]. The recommended first-line treatment in published guidelines in Europe and North America reflect this with PPI combined with amoxicillin and clarithromycin being the favored regimen [1,4]. However, some caveats have been applied to these guidelines in recent years to take into account falling eradication rates. As recently as 2000, studies had suggested eradication rates for standard triple therapy were in excess of 90% [5]. However, more recent publications have suggested that this level has fallen alarmingly to be around 70% in many areas and even as low as 60% in some [6,7]. For instance, the most recent Maastricht guidelines recommend substituting metronidazole for clarithromycin where resistance to that antibiotic exceeds 15–20% [3]. Eradication rates with this regime are 87.8% when strains are clarithromycin sensitive and 18.3% when strains are clarithromycin resistant [8]. The rate of clarithromycin resistance is increasing, probably due to greater use of clarithromycin in the community for respiratory tract infections [9,10]. An Italian study noted that rates of clarithromycin resistance increased twofold in that country from 1990 to 2005 [11]. A similar phenomenon was noted in England with resistance rates rising by 57% between 2002 and 2006 [12]. A study in the United States estimated clarithromycin resistance at 10.1% [13]. There has also been debate as to the ideal duration of therapy. In recent
years, longer regimes have supplanted the previous 7-day triple therapy. A meta-analysis in 2000 suggested a 14-day course of therapy showed 7–9% better cure regimens than 7-day regimes [14]. However, another meta-analysis differed, stating no clinical benefit from longer courses of treatment, although the quality of some of the studies included in this second meta-analysis has been questioned [15]. The published guidelines are also beginning to reflect this with the 2007 American College of Gastroenterology guidelines recommending 10-day treatment courses [4]. In addition, the most recent Maastricht consensus stated that 14 days of treatment had an advantage over 7 days in terms of eradication [1].

Second-line Therapy

As first-line therapy has been noted to fail in approximately 20% of patients, the need for effective second-line therapy is clear [16]. Many putative second-line therapies are currently in use but the most common are bismuth-based and levofloxacin-based therapies [17]. Bismuth-based quadruple therapy consisting of a PPI, bismuth, tetracycline, and metronidazole is reported to have an efficacy of 76% in patients who failed first-line therapy [18]. This is generally given for 10 days and taken four times daily, although a study of a 14-day twice daily regimen reported 95% efficacy in a mix of first-line and second-line patients which might improve compliance and tolerability [19]. Bismuth-based therapy has proved quite safe. A meta-analysis in 2008 showed no serious adverse event in 4763 patients who received it. No statistically significant increase in any side effect other than dark stools was illustrated [20]. Levofloxacin-based therapy has grown in popularity in recent years. A very recent Spanish multicenter study of 300 patients who had failed first-line eradication therapy revealed 81% per-protocol and 77% intention-to-treat analysis when a 10-day levofloxacin-based regimen was used, with good tolerability and a low side effect rate of 22% [21]. Concerns have been expressed regarding the development of fluoroquinolone resistance when levofloxacin is used for Helicobacter eradication. A rapidly increasing rate of quinolone resistance was reported in several countries: 15% in 2004 in Japan, 16.8% in 2006 in Belgium, from 11.2% in 2003 to 22.1% in 2005 in Germany, from 3% in 1999 to 15% in 2004 in France and from 2.8% to 11.8% between 1998 and 2003 in Taiwan [22–28]. The apparently rapid rate at which fluoroquinolone resistant seems to develop may limit the use of levofloxacin in H. pylori eradication to second-line therapy. Another concern exists regarding the side effects of the fluoroquinolones. Tendonitis was reported in 704 of 46,000 patients receiving levofloxacin in one study [29]. Other case reports have noted hepatotoxicity [30].

Third-line Therapy

Patients who fail both initial- and second-line therapy for H. pylori pose an interesting and challenging question [31]. Compliance must, of course, be questioned. The options are to use further empiric regimes or to employ treatments tailored to individual antibiotic sensitivities. Two of the more common empiric rescue antibiotics used are rifabutin [32] and furazolidone [33]. Rifabutin is an antituberculous agent. For the eradication of H. pylori, it can be administered as PPI, rifabutin (150 mg), amoxicillin (1 g), all twice daily for 14 days. One study on rifabutin used for treatment failures achieved 95% eradication rates as second-line therapy and 68% eradication for third- or subsequent line therapy [34]. Another study limited to patients who did not achieve eradication with standard first-line or bismuth-based second-line therapy revealed 79% eradication rates based on intention-to-treat analysis [35]. These results, however, have been contradicted somewhat by the largest study to date on rifabutin as a third-line treatment which estimated eradication rates as being 61% [36]. Rifabutin is limited as a treatment option by a number of factors. Stocks are low in Europe. Also, rifabutin is a useful tool in the treatment of the increasingly problematic multi-drug resistant tuberculosis infection. Greater use of rifabutin in the treatment of H. pylori would likely result in the development of more resistant strains of Mycobacterium tuberculosis. Also serious myelotoxicity and ocular adverse events have been reported with this treatment [37,38]. Furazolidone is also useful in treatment failures [39,40]. A study of 10 patients, in whom first-line, second-line and rifabutin-based therapy had failed revealed 60% eradication when it was used along with amoxicillin and PPI [41]. When this data was incorporated into a systematic review of furazolidone-based treatments for third- and subsequent line eradication therapy, they were shown to be effective 65% of the time [42]. The other principal strategy for salvage therapy in H. pylori involves cultural and antibiotic testing. This is a very logical approach as H. pylori is a latent infection and therefore has more in common with other latent conditions such as tuberculosis and syphilis, where susceptibility testing is routinely employed, than with conditions, such as a urinary or a respiratory tract infection where empiric antibiotic regimes are used. Susceptibility testing is limited by the fact that in vivo resistance may not accurately reflect in vitro resistance, notably with respect to
Sequential Therapy

Sequential therapy has been proposed as an alternative to standard triple therapy for the eradication of H. pylori [46,47]. The primary goal of this regimen is to overcome clarithromycin resistance. Hypothetically, during the first part of therapy, amoxicillin weakens the bacterial cell wall, which prevents the formation of the channels that block clarithromycin from binding to the bacterium and hence causes resistance to the antibiotic. A meta-analysis published last year demonstrated that eradication rates with sequential therapy are 93.4% compared with 76.9% for standard triple therapy [48]. Sequential therapy is not affected by bacterial factors (CagA status, bacterial load) and host factors (underlying disease, smoking) which, until now, have predicted the outcome of conventional eradication treatments. Even when strains were clarithromycin resistant, the eradication rate with sequential therapy was 82.2% compared with 40.6% for triple therapy. So far, almost all of the studies analyzing sequential therapy have been performed in Italy and the sequential regimen has been given equivalent status to standard 7–14 day triple therapies as first-line treatment in the updated Italian guidelines on H. pylori management, where it has been stated that: “The Working Group advised the use of 7–14 day triple therapies or a sequential therapy as first-line treatment” [49]. The main drawback to sequential therapy may lie in its complexity and how this may affect compliance. Although the meta-analysis quoted showed that compliance was superior amongst patients receiving sequential therapy compared to standard triple therapy, it is counter-intuitive that a regimen which lasts longer and involves a change in the medications consumed in mid-course could enhance compliance [50]. The advantages of sequential treatment over triple-therapy need to be confirmed in randomized controlled trials in different countries and settings before a generalized change is recommended in first-line H. pylori treatment. Accordingly, the American College of Gastroenterology Guideline on the Management of Helicobacter pylori Infection states that “Sequential therapy may provide an alternative to clarithromycin-based triple therapy but requires validation within the United States before it can be recommended as a first-line therapy” [4], and the European Maastricht III Consensus Report points out that “Sequential treatment deserves further evaluation in different regions” [1]. The main disadvantage of the sequential therapy regime is that it is more complex for the patient, requiring a change of medication in the middle of the treatment period. Although it was not shown in the meta-analysis, it is felt that this would likely have a negative impact on compliance. Whether it is necessary to provide the drugs sequentially or whether the four constituent components of sequential therapy can be given concurrently is unclear. A meta-analysis published in 2009 on this showed a per-protocol eradication level of 92.9% and intention-to-treat eradication of 89.7% [51]. This quadruple therapy appears to be an effective, safe and well-tolerated alternative to triple therapy and is less complex than sequential therapy, emphasizing that studies comparing both alternatives are urgently needed. It must be noted that although it is designed to overcome clarithromycin resistance, clarithromycin is central to both sequential and quadruple therapy and would still be at the mercy of changes in patterns of clarithromycin resistance which are probably primarily contingent on the rates of prescription of clarithromycin in the community for nongastrointestinal infections [52]. In addition, there exists a body of opinion that clarithromycin and metronidazole ought not be used together for H. pylori eradication as those who fail to have eradication will subsequently have at least single and often double resistance [53]. Sequential therapy undoubtedly shows promise but must be further evaluated before it can supplant triple therapy in the existing guidelines.

Adjuncts

Adjunctive therapies may offer some promise in H. pylori eradication. Probiotics have been proposed as a useful adjunct [54]. In one study undertaken in 2008, prescribing probiotics with H. pylori eradication therapy had no effect on the side effect profile but did increase the rates of eradication [55]. However, another study on concurrent probiotic administration suggested the inverse with better side effect profiles but no increase in eradication or rates of compliance with therapy [56]. Vaccination has also been proposed as a means of controlling H. pylori and the morbidity associated with it. The challenge model was first established in human volunteers in 2004 [57] and subsequent to this, a vaccine is in Phase I trials and its manufacturers claim it has been shown to be safe and immunogenic in early trials [58].
Conclusion

Establishing efficacious and acceptable treatment regimens for patients infected with *H. pylori* continues to pose problems for physician and patient alike. The decrease in eradication rates needs to be firmly addressed with evidence-based clinical practice. It is probably the case, however, that the tools to successfully eradicate *H. pylori* are already present and that they simply need to be properly utilized. It has been repeatedly illustrated that, if compliance is good and a clear treatment paradigm adhered to, very high eradication rates can be achieved. For example, a study published in 2008 in a Finnish tertiary referral centre revealed 100% eradication in 644 consecutive patients where compliance was ensured and patients were treated with standard first- and second-line therapies as per the Maastricht guidelines and third-line rescue therapy was tailored to antibiotic susceptibility [59]. Another study in Greece published earlier this year found 98.1% eradication rates when the Maastricht-III guidelines were implemented with empiric therapy used for third-line patients [60]. Another 2008 study evaluated the efficacy of different ‘rescue’ therapies empirically prescribed during 10 years to 500 patients in whom at least one eradication regimen had failed to cure *H. pylori* infection. The authors concluded that it is possible to construct an overall treatment strategy to maximize *H. pylori* eradication, on the basis of administration of four consecutive empirical regimens [61]. The key factors in these studies were an awareness of the importance of compliance and the provision of structured aftercare and follow-up programs to ensure eradication. It has been proven that such measures can improve compliance [62]. It is very likely the case that empowering patients to achieve high levels of compliance is what accounts for the impressive eradication rates in centers where patient follow-up is structured and comprehensive [63]. While it is important to develop new regimens to overcome the problems of resistance, a need also exists to work as efficiently as possible with our current regimes and facilitate patient compliance. If compliance is not achievable, the problem of antibiotic resistance will continue to beset any combination of drugs used for *H. pylori* eradication.

Conflicts of Interest

The authors have declared no conflicts of interest.

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Treatment of H. pylori infection

O’Connor et al.


**Helicobacter pylori Infection in Pediatrics**

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**Keywords**

Children, prevalence, recurrent abdominal pain, anemia, stool antigen test, treatment.

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**Abstract**

This review summarizes the articles published on *Helicobacter pylori* infection in children between April 2008 and March 2009. Recent evidence highlights the decreasing prevalence trend of *H. pylori* infection and supports both intrafamilial and extrafamilial transmission. The association with various symptoms is still being debated. Interestingly, *H. pylori* infection seems inversely associated with allergic diseases. Monoclonal stool antigen tests are widely used and accurate for the diagnosis of *H. pylori* infection, but less accurate in young children. The new biprobe real-time PCR assay applied to stools showed a poor sensitivity in children. Using the urea hydrolysis rate next to the delta over baseline values, the $^{13}$C-urea breath test provides excellent results for all age children, even for young children. Treatment of *H. pylori* infection remains a challenge, considering suboptimal efficacy of current therapy. Among emerging alternatives, sequential treatment appears promising. The adjunction of probiotics to conventional regimens, although eliciting great interest, has shown limited therapeutic benefit.

**Pathogenesis**

Whereas there is evidence for a role of genetic markers on disease severity in adults, in children this association is not clear. Ko et al. found no association between cagA, vacA, and iceA with gastritis severity in Korea [1]. On the other hand, Oleastro et al. suggested the homB gene as a new putative virulence marker highly associated with peptic ulcer disease (PUD) in children and adults. HomB also correlated with the presence of cagA, babA2, vacAs1, hopQ1, and oipA and seemed involved in *Helicobacter pylori* adherence and in inflammatory response [2].

To determine whether host gene polymorphisms coding for Toll-like receptors (TLR) influence the immune response to the infection, Moura et al. studied TLR2, TLR4, and TLR5 polymorphisms in a large cohort of children. None of them were associated with *H. pylori* infection or duodenal ulcer disease. Otherwise, the presence of TLR4 was associated with infection by cagA-positive strains and with increased levels of interleukin (IL)-8 and -10 [3]. This might contribute to more severe consequences of the infection in adulthood. A Polish group found significantly higher IL-1β, IL-8, and IL-1β transcript levels and macrophage number in the antral mucosa of *H. pylori*-infected children than in *H. pylori*-negative children. They also observed a significant correlation between macrophage number and histological parameters of gastritis [4]. Another group from Turkey found that expression of $\alpha$-defensin was significantly higher in *H. pylori*-infected children and associated with higher grades of inflammation and neutrophil density [5]. Leach et al. from Canada showed a high expression of the inflammatory S100 calgranulin proteins in *H. pylori*-infected mucosa, correlating with the severity of gastritis, and its absence in normal gastric mucosa [6].

Czaja et al. found that the mean fasting serum gastrin level was higher in children with *H. pylori*-associated gastritis compared to *H. pylori*-negative cases, with or without gastritis. In contrast to earlier studies suggesting that a decrease in the number of somatostatin-producing D cells with an unchanged number of gastrin-producing G cells may lead to PUD, these authors found no difference in G-cell density and D-cell density, regardless of the presence or absence of gastritis or *H. pylori* infection [7].

**Prevalence**

A cross-sectional population-based study of *H. pylori* infection prevalence was conducted on 2480 Chinese
children (age 6–19 years) by using \(^{13}\text{C}\)-urea breath test (UBT) [8]. A surprisingly low prevalence rate was found, with an overall positivity of 13.1%, related to a low educational level of the child’s mother (OR = 2.43), family history of gastric cancer (OR = 2.19), and household member number >5 (OR = 1.57).

Siai et al. reported a 51.4% seroprevalence rate (ELISA IgG) in Tunisian school children, 6 years old, significantly related to household crowding, late bottle-weaning, and bed-sharing [9].

Two studies evaluated \(H.\ pylori\) infection prevalence by using a stool antigen test. Kori et al. observed a prevalence rate of 24.7% in daycare children from Israel with higher rates in the 13- to 60-month-old group (32.5%) compared to the 3- to 12-month-old group (7.1%), suggesting infection acquisition most probably after the first year of life [10]. Yuvel et al. showed a 30.9% prevalence rate in asymptomatic Turkish children (mean age 6.8 ± 3.0 years), related to a low education level of child’s mother, adverse living conditions, and a high number of siblings [11].

In India, Mishra et al. confirmed an increasing detection rate of \(H.\ pylori\) with age, by documenting positivity in saliva/stool samples by nested PCR in 2.1%/4.25% (<5 years), 22.7%/13.6% (6–10 years), 55.9%/50% (11–16 years) of cases, respectively [12].

Three retrospective observational studies assessed \(H.\ pylori\) infection prevalence in children submitted to endoscopy. Elitsur et al. observed an overall infection rate of 12.1% and a significant decrease in mean annual infection rate in the last 6 years of the study period in 1743 North American children over a 13-year study period [13]. A decreasing prevalence from 60.4% to 30.4% (first and last years of the study, respectively), was reported by Kawakami et al. in Brazilian children over a 10-year period [14]. In Canada, Segal et al. documented a very low overall infection rate (7.1%) [15].

Transmission

The transmission of \(H.\ pylori\) remains poorly understood. New data including fingerprinting analysis studies, supports both intrafamilial (mostly mother-to-child) and extrafamilial transmission. In a systematic review which included a birth cohort study enrolling 1066 healthy newborns, Weyerman et al., using a monoclonal stool antigen test, identified maternal infection as the single significant risk factor (OR 13.0) for acquisition of infection in childhood [16]. Konno et al., using DNA fingerprinting analysis of cultured \(H.\ pylori\) from 42 children and their infected family members, identified fingerprint patterns identical to those of at least one family member in 76% of the children, with a significantly higher rate of identity in the mothers’ patterns, compared to those of fathers (\(p < .01\)). Mother-to-child transmission was thus suggested as the most probable route of transmission of \(H.\ pylori\) [17].

Herrera et al. compared \(H.\ pylori\) genotypes (cultures and/or DNAs obtained by the string test) from members of low income families in Peru [18]. Interestingly, in 70% of the cases, mother–child strain pairs did not match, nor did most strains from siblings or other family members, thus further suggesting the possibility of community acquisition of \(H.\ pylori\) infection.

Symptoms

The association with many symptoms is still a subject of great debate.

Recurrent Abdominal Pain

During the last year, there was just one study concerning this topic. In agreement with most previous studies, Masoodpoor et al. found no relationship between recurrent abdominal pain and \(H.\ pylori\) infection in children. The prevalence of \(H.\ pylori\) infection in children with RAP and in healthy children in the age range of 12–15 years was similar [19].

Peptic Ulcer and Reflux Disease

Houben et al. analyzed retrospectively 76 patients who were admitted to the hospital with signs of acute upper gastrointestinal bleeding. \(Helicobacter pylori\) was identified in 55% of these patients and in 90% a duodenal ulcer was found. This shows a strong relation between gastrointestinal bleeding because of duodenal ulcer disease and \(H.\ pylori\) infection in childhood [20].

There were no new studies regarding gastroesophageal reflux disease nor nonulcer dyspepsia during this period.

Extra-gastrointestinal Manifestations

During this period many studies continued to investigate the relationship between \(H.\ pylori\) infection and extraintestinal manifestations such as iron deficiency anemia (IDA), growth failure, asthma, atopy and various other conditions.

Iron Deficiency Anemia

Fagan et al. concluded that \(H.\ pylori\) plays a casual role in hematological outcomes of children. They followed children after \(H.\ pylori\) eradication treatment for a
period of 40 months. They found a lower prevalence of iron deficiency and IDA in *H. pylori*-negative children compared with *H. pylori*-positive children [21]. On the other hand, no association between *H. pylori* infection and IDA was seen in three other studies [22–24]. It is indeed difficult to distinguish between anemia due to infection with *H. pylori* and to other confounding factors, such as poor nutritional status or another underlying disease.

**Growth Failure**

There is ongoing discussion about the association between *H. pylori* and growth retardation. In the past, possible mechanisms such as malabsorption and decreased appetite were mentioned as a possible cause of growth failure. During the last year, two studies were added on this subject. Both papers did not support a role of *H. pylori* on growth failure in children [25,26].

**Allergy**

In developed countries, allergies have become more prevalent in recent decades, whereas the prevalence of *H. pylori* has been decreasing in these countries. This gives rise to search about the association between this micro-organism and allergies.

Chen & Blaser carried out a cross-sectional analysis on the data from 7412 pediatric participants in the National Health and Nutrition Examination Survey (NHANES) in the U.S., to assess the association between *H. pylori* infection and childhood asthma. They found that *H. pylori* seropositivity was inversely associated with asthma, recent wheezing, allergic rhinitis, dermatitis, eczema, and rash [27]. A second study confirmed a low rate of *H. pylori* antibodies in children with bronchial asthma [28]. These findings are intriguing and incite thought about research in this field, including asthma prevention. Cam et al. investigated immune responses (T-helper cell function) in *H. pylori* infected children and compared the cytokine responses in the atopic and nonatopic group. The frequency of atopy was lower in the *H. pylori*-infected group (31.9 vs 48.1%), whereas atopic symptoms were similar between infected and noninfected children. Their results demonstrated a counteractive cytokine interaction between *H. pylori* infection and atopy, but it did not protect against atopy [29].

**Atherosclerosis**

*Helicobacter pylori* infection has been proposed to play a role in the development of atherosclerosis preceded by endothelial dysfunction. Coskun et al. found no early findings of atherosclerosis in *H. pylori* infected children using noninvasive techniques such as Doppler ultrasonography [30].

**Diagnostic Tests**

Besides invasive diagnostic methods (histology, culture, and rapid urease testing following endoscopy) which are still considered the “gold standards,” there are still no 100% specific and sensitive noninvasive tests for the diagnosis of *H. pylori* in children. Especially in infants, noninvasive tests are less accurate. In a high prevalence country, such as Turkey, 26.3% of all children younger than 2 years of age who underwent endoscopy were *H. pylori*-positive. Most of them (65%) already showed histopathologic abnormalities such as gastritis [31].

In 2005, the Canadian Consensus group concluded that 11C-UBT is the best available and most reliable noninvasive test in children, but it is far less accurate in younger children [32]. Two studies on UBT were published during the last year measuring the urea hydrolysis rate (UHR) next to the delta over baseline values (DOB). Both were able to show that using UHR next to DOB provided excellent results for children of all ages, resulting in less false positive results in children under the age of 6 years [33,34].

Monoclonal stool antigen tests are widely used and accurate for the diagnosis of *H. pylori* infection in children, but their use in young children remains controversial. Ritchie et al. found a very low sensitivity (55%) and specificity (68%) in children from 4 months to 2 years [35]. Other studies have investigated the accuracy of rapid immunochromographic stool antigen tests (Rapid HpSA) with discrepant results. While Yang et al. and Cardenas et al. found high sensitivities (94.6 and 100%) and specificities (98.4 and 100%) [36,37], Kuloğlu et al. found far less accurate results (pre- and post-treatment: specificity 92.3 and 100%, respectively; sensitivity 65 and 60%, respectively) [38]. Whereas in adults the new biprobe real-time PCR assays applied to stools showed excellent results, Falsafi et al. found a reasonable specificity of 92.3%, but a poor sensitivity of 62.5% in children. However, this study also noted an association between higher scores of *H. pylori* in histology and more severe gastritis with positivity of stool PCR [39]. This could explain the insufficient sensitivity in children who, for the most part, have less severe gastritis.

In addition to several studies published on serology, Leal et al. published a meta-analysis on antibody-based detection tests for the diagnosis of *H. pylori* in children [40]. ELISA-IgG assays showed low sensitivity (79.2%)
but good specificity (92.4%). Commercially available ELISA tests varied widely in performance. Western blot tests showed a good overall performance (sensitivity 91.3% and specificity 89%). In-house ELISA with whole-cell antigen tests showed the highest overall performance (sensitivity 94% and specificity 96.4%). This review showed the need for an evaluation of the serological test in the community in which it is be used.

**Treatment**
Francavilla et al. showed for the first time the superiority of a 10-day sequential treatment in children compared to standard treatment [41]. An overall eradication rate of 85.2% was obtained, irrespective of the presence of ulcer or cagA status [42].

A single-centre study from Turkey using a first-line standard therapy (amoxicillin, clarithromycin and proton pump inhibitor (PPI)), did not show any significant impact of the duration of treatment on eradication rate (per-protocol analysis), 55.8 and 60.5%, in the 7- and 14-day group, respectively [43]. Furthermore, in the subset of nonresponders, a second-line quadruple therapy protocol comprising bismuth citrate, doxycycline, metronidazole, and PPI (7 days) obtained a 64.6% eradication rate. The contribution of a high background resistance to clarithromycin and metronidazole to the low global eradication rates obtained was admitted.

Nguyen et al. compared the efficacy of two 14-day triple regimens including amoxicillin, lansoprazole and clarithromycin or metronidazole in a randomized double-blind trial and observed an overall per-protocol eradication performed in Vietnam similarly low in both regimens, 62.1 and 54.7%, respectively [44].

Caristo et al. applied the fluorescent in-situ hybridization test on pediatric gastric biopsy specimens during two consecutive 5-year periods [45]. The study confirmed high sensitivity and specificity for the co-detection of sensitive and resistance strains, further showing that in one-third of the cases with mixed infection, resistant strains were only seen in the fundus. This emphasizes the relevance of fundus biopsies.

Probiotics as alternative therapeutic options have recently emerged. Until 2006, four controlled trials evaluated the contribution of lactic acid bacteria and *Saccharomyces boulardii* to *H. pylori* eradication and reduction of treatment side-effects in children, with conflicting results [46]. Hurduc et al. compared the efficacy of standard eradication therapy (PPI, amoxicillin and clarithromycin) for 7–10 days, plus *S. boulardii*, 250 mg b.i.d. for 4 weeks (intervention group) with standard eradication therapy alone (control group) [47]. Although the addition of *S. boulardii* to the standard treatment offered only a 12% additional therapeutic benefit (eradication rate of 80.9% in the control group vs 93.3% in the intervention group) it significantly reduced the incidence of side effects (30.9 vs 8.3%, respectively).

Gottelaud et al. assessed the potential additive or synergistic effect of *Lactobacillus johnsonii* La1 plus cranberry juice on the inhibition of *H. pylori* in a multicenter, randomized, controlled, double-blind trial (3 weeks), including asymptomatic children with infection confirmed by UBT. Eradication rates were significantly lower in the control group (placebo juice /heat-killed La1) (1.5%), compared to the intervention groups (14.9, 16.9, and 22.9% in the placebo juice/La1, cranberry juice/heat-killed La1 and cranberry juice/La1 groups, respectively) [48], showing the absence of synergistic inhibitory effects of La1 plus cranberry on *H. pylori* colonization.

Re-infection after successful eradication has also received increased attention, as rates from 1.9 to 9.6% have been reported in children, mostly attributed to interfamilial transmission, with higher rates in developing countries [49].

**Conflicts of Interest**
The authors have declared no conflicts of interest.

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Since the late 1980s, several studies have reported the link between chronic *Helicobacter pylori* or Helicobacter species infection and a variety of extragastric manifestations. These include ischemic heart disease (IHD), liver diseases, skin diseases, blood disorders, and others [1]. For several of these supposed associations, the hypothesis of a causal role has yet to be fully investigated. This may be due to a series of factors linked to the epidemiological features of the studies and to the diseases investigated. This review attempts to highlight the main reported associations of *H. pylori* with extragastric manifestations during the last year. The most convincing data arise in the field of idiopathic thrombocytopenic purpura (ITP) and sideropenic anemia. Long-term follow-up studies have shown that 50% of subjects with ITP maintain a hematological response after *H. pylori* eradication. There is also growing evidence of the role of *H. pylori* in other diseases, including ischemic heart disease even though results are not conclusive.

### Heart and Vascular Diseases

#### Atherosclerotic Disease: IHD, Stroke, and Peripheral Arterial Involvement

Two aspects of *H. pylori*, *H. pylori* involvement in atherosclerotic disease were investigated: epidemiology and pathogenesis. Regarding IHD, Aiello et al. evaluated the socioeconomic and psycho-social gradients of pathogen burden of four infectious agents (cytomegalovirus, herpes simplex virus-1, *H. pylori* and *Chlamydia pneumoniae*). By including 999 healthy adults (mean age 59 years) selected from the longitudinal study MESA (Multi-Ethnic Study of Atherosclerosis) aimed at identifying risk factors for atherosclerosis, the authors showed that low education (odds ratio (OR) 95%, confidence interval (CI): 1.37, 1.19–1.57) and a higher level of chronic psycho-social stress were significant independent predictors of higher pathogen burden after adjustment for covariates [3]. In a study from Turkey, the authors focused on the seroprevalence of antibodies to *H. pylori* in 73 patients with acute coronary syndrome, in 79...
patients with chronic stable angina, and in 22 control subjects. They showed a significantly higher rate of positivity in patients than in controls (80.2 vs 54.5%, p = .015). This difference was not evident between the two groups of patients (86.3 vs 74.6%, respectively, p > .05). C-reactive protein (CRP) was higher in subjects with acute coronary syndrome than in those with stable angina. However, no adjustment for socioeconomic factors was made [4]. Similar results were reported in India, where the seroprevalence of IgA and IgG to H. pylori was significantly higher in 192 patients with an incident or prevalent IHD with respect to 192 age- and sex-matched controls (for both p < .001). The level of CRP was higher in subjects positive for IgA, but not for IgG to H. pylori. On the basis of these findings, the authors proposed that the association of CRP with IgA to H. pylori be used as marker to target the population at high risk for IHD [5]. Opposite results were found in Iran where only serum antibodies to C. pneumoniae were associated with late cardiac events [6]. The study by Nikolopoulou et al. supported the association between seropositivity for anti-H. pylori IgG and coronary atherosclerosis, but not in its acute phase. Furthermore, a potential causal role involving the overexpression of tumor necrosis factor alpha (TNFα) and vascular cell adhesion molecule-1 is not supported by data [7]. To clarify if more virulent H. pylori strains (expressing the CagA antigen) were involved in coronary instability, Franceschi et al. performed a clinicopathological study and a meta-analysis on 4241 cases. In their study, the authors showed that the anti-CagA antibody titer was significantly higher in patients with unstable angina compared to those with stable angina (p < .02), normal coronary arteries (p < .01) or healthy controls (p < .02). Moreover, anti-CagA antibodies recognized antigens localized inside coronary atherosclerotic plaque in all specimens from both stable and unstable patients. In the meta-analysis, seropositivity to CagA was significantly associated with the occurrence of acute coronary events (OR: 1.34, 95%CI, 1.15–1.58, p = .0003) [8]. These findings support the potential role of more virulent H. pylori strains in the acute phase of IHD, a pathogenic model postulated on the basis of previous observations [9], and are not mutually exclusive with the association of the infection with increased circulating low-density lipoprotein cholesterol and triglyceride levels [10]. The implications of more virulent strains have been confirmed by a systematic review on 15 studies, which showed an OR: 2.11 (95% CI: 1.70–2.62) for CagA seropositivity in the development of IHD [11]. Against a local implication in IHD [9], there is the evidence that, contrary to C. pneumoniae, Mycoplasma pneumoniae, herpes simplex, and cytomegalovirus [12], the infrequent detection of H. pylori DNA in the plaque or in human arteries is possible [13,14]. Finally, Honda et al. showed that H. pylori infection does not accelerate the age-related progression of arteriosclerosis in a 4-year follow-up [15].

Regarding stroke, in a primary care multicenter study, a trend toward a higher prevalence of active H. pylori infection in patients with ischemic stroke with respect to controls (63 vs 54%) has been found. This difference became significant when focusing on more virulent strains (OR: 2.69, 95% CI: 1.37–5.30) [16]. This is in agreement with another study that an infection with CagA-positive H. pylori strains increases the risk of recurrent atherosclerotic stroke with a hazard ratio: 3.5 (95% CI: 1.9–6.4; p < .001) [17]. In the review reported above, Zhang et al. found that the OR of ischemic stroke for more virulent strains in 11 studies was 2.68 (95% CI: 2.20–3.27) [11]. In a prospective study, performed in Pakistan on 326 subjects with a 2-year follow-up, three patients with active H. pylori infection and one without infection had a stroke or transient ischemic attack [18]. This difference was not statistically significant. According to the authors larger, prospective, randomized studies are needed.

Regarding other arteriopathies, Sawayama et al. showed that the prevalence of H. pylori infection was significantly higher in patients with peripheral arterial disease than in controls (79.7 vs 44.8%, p < .01) [19]. Nyberg et al. investigated, with negative findings, whether seropositivity for antibodies to H. pylori, or to a burden of pathogens, including also C. pneumoniae, cytomegalovirus, and herpes simplex virus, were related to abdominal aortic aneurysm rupture [20]. In a preliminary investigation, the authors found a relationship between H. pylori infection and cardiac syndrome X [21]. This encourages more robust studies.

The involvement of the bacterium in metabolic disturbance or inflammatory processes potentially related to atherosclerotic disease has been explored. Gunji et al. reported the seroprevalence of H. pylori infection in healthy Japanese adults with and without metabolic syndrome. A total of 38.6% among the former vs 28.0% among the latter (p < .001) were positive for H. pylori antibodies. Furthermore, a number of metabolic syndrome components (high systolic blood pressure, low HDL-cholesterol, and high LDL-cholesterol levels) were found significantly associated with H. pylori seropositivity by multiple linear regression analysis [22]. Helicobacter pylori has also been shown to affect the vascular risks and complications in patients with diabetes mellitus although data concerning the prevalence of H. pylori infection among these patients are scanty and controversial [1]. Hamed et al. evaluated...
the prevalence of *H. pylori* infection in patients with diabetes mellitus, the association between the former and diabetic vascular complications, and the influence of the bacterium on atherosclerosis and inflammatory biomarkers. The prevalence of *H. pylori* infection was higher in patients compared to healthy controls. Carotid artery intima-media thickness and inflammatory biomarkers, as interleukin (IL)-6 and TNFα, were significantly higher in infected patients. In the multivariate analysis, blood glucose, triglycerides, erythrocytic sedimentation rate, IL-6 and TNFα increased the OR for atherothrombotic cause of cerebral ischemia in patients with *H. pylori* infection [23]. In a study performed in Japan, Ohnishi et al. showed in 130 patients with type 2 diabetes mellitus, without history of cardiovascular disease, that *H. pylori* infection was associated with arterial stiffness determined by pulse wave velocity [24].

**Arrhythmias**

Besides IHD, the possible association between *H. pylori* infection and atrial fibrillation has been previously published. In the last year, Platonov et al. reported, in a case–control study, that permanent atrial fibrillation is associated with elevated CRP levels, but the latter is not the result of earlier infection with *H. pylori* or *C. pneumoniae* [25]. This is in agreement with the conclusion of an editorial that, in light of the existing results, the responsibility of *H. pylori* infection has been excluded in the development of atrial fibrillation [26].

**Respiratory and Ear, Nose, and Throat (E.N.T.) Diseases**

A wide spectrum of manifestations linked to *H. pylori* has been reported over the last year. Regarding asthma, a study has shown that in children, the rate of *H. pylori* antibodies is low and a significant difference could not be detected in gastroesophageal reflux disease and atopy between patients negative and positive for *H. pylori* [27]. These findings are in agreement with those of a cross-sectional analysis on 7412 participants in a U.S. National survey. In this case, the authors showed that *H. pylori* was inversely associated with a history of asthma (95% CI, OR: 0.69, 0.45–1.06) [28]. Data from another U.S. study on 318 patients and 208 controls, also indicated that infection with more virulent strains is inversely associated with asthma (95% CI, OR:0.57, 0.36–0.89) and is associated with an older age of asthma onset [29]. In an animal model, *H. pylori* neutrophil-activating protein (HPNAP) reduced eosinophilia, IgE, and Th2 cytokine levels in bronchoalveolar lavage [30]. Whether HPNAP is a candidate for novel strategies of prevention and treatment of allergic diseases remains to be elucidated. In contrast, in a study on 2437 randomly selected adults, Fullerton et al. failed to demonstrate an association between *H. pylori* exposure and chronic obstructive pulmonary disease, measures of allergic disease or decline in lung function [31].

Colonization by *H. pylori* of the larynx has been shown by Titiz et al. detected *H. pylori* DNA by PCR in 17 of 21 samples of patients operated on for laryngeal squamous cell carcinoma. This DNA was present both in normal and in tumoral tissue (76.2 vs 42.9%, *p* = .039). On the contrary, *H. pylori* DNA was not found in samples of 19 patients with benign laryngeal pathology (*p* = .0001) [32]. Grbesa et al. also detected *H. pylori* by Giemsa staining and nested-PCR in 13 of 82 (16%) samples from patients with laryngeal squamous cell carcinoma [33]. Rezaei et al. showed that seropositivity for antibodies to *H. pylori* was significantly associated with laryngohypopharyngeal carcinoma [34]. In an evidence-based meta-analysis, including five case–control studies, laryngeal cancer risk for patients with *H. pylori* infection was 2.03-fold higher (95% CI 1.28–3.23) [35].

Zycinska et al., on the basis of a study on 36 patients with pulmonary Wegener’s granulomatosis disease, suggested that disease severity, prevalence of gastroduodenal lesions, and type and duration of treatment is dependent upon *H. pylori* infection [36].

Regarding E.N.T. Fancy et al. observed, in pediatric patients with otitis, more *H. pylori* DNA in adenoids of patients than in those of controls (10 of 45 vs 6 of 37, respectively, *p* = .49) [37]. *Helicobacter pylori* whole-cell protein directly induced a macrophage migration inhibitory factor, macrophage inflammatory protein 2, IL-1β, and TNFα in middle ear epithelium in experimentally infected mice. Moreover, severe proliferation of inflammatory cells was observed in the middle ear cavity inoculated with *H. pylori* whole-cell protein [38]. In a critical evaluation of the evidence of the relationship between *H. pylori* and otitis media with effusion, the authors, after examination of six original papers, with a total of 203 patients and 27 controls, concluded that there is actually poor proof of correlation [39].

Using urease test, Eyigor et al. found *H. pylori* positivity in three of 55 adenontonsillar tissue specimens of patients with adenotonsillitis; however, none was positive when analyzed by PCR [40]. Similar results were reported in pediatric patients with chronic tonsillitis, in whom *H. pylori* did not colonize tonsil tissue [41].

Uncertainties persist concerning the association between *H. pylori* and nasal polyps. While Cvorovic et al. found *H. pylori* in six of 23 specimens by urease
test and by histochemical analysis with Giemsa staining [42]. Ozcan et al. detected *H. pylori* only in one of 25 specimens by each of these methods [43]. By using a questionnaire, another group has shown, in a prospective study that *H. pylori* eradication chronic nonspecific pharyngeal symptoms [44] but the mechanism of this benefit should be better investigated. It probably depends on acid inhibition rather than bacterium cure per se. In fact, in the work by Toros et al., all patients responded well to anti-reflux treatment but no correlation was observed between *H. pylori* positivity and symptoms [45]; in the study of Oridate et al., acid-suppression therapy offered slower laryngopharyngeal than esophageal symptom relief in laryngopharyngeal reflux patients, and these differences were observed independently from *H. pylori* status [46].

**Hematologic Diseases**

**Idiopathic Thrombocytopenic Purpura (ITP)**

After the pioneer report by Gasbarrini et al. [47], the association between *H. pylori* and ITP obtained a formal recognition in the Maastricht III Consensus report which recommended that *H. pylori* infection should be sought after and treated in patients with ITP [48].

During the last year, a Canadian prospective study showed that in subjects with ITP, 48 months after *H. pylori* eradication, 75% achieved a complete or a partial response and 50% had a long-term ongoing response [49]. Unfortunately, the small sample size (four *H. pylori*-positive patients) limits the value of the long-term follow-up. In a 7-year follow-up prospective study conducted in Japan and including 30 subjects, *H. pylori* eradication had a short-term efficacy in about 58–68% of patients [50]. In Korea, in patients who did not respond to steroid and/or danazol therapy for ITP, a combination therapy consisting of *H. pylori* eradication plus immunosuppressive therapy induced, after 6 months, a statistically higher response than *H. pylori* eradication alone (66.7% vs 41.7%, *p* = .345). Furthermore, the median response duration was also longer in the former than in the latter group (9 vs 3 months, *p* = .049) [51]. In contrast, in Australia, four of nine ITP patients receiving eradication treatment showed no response and underwent splenectomy, and one relapsed after 3 months [52]. In a systematic review, original articles reporting 15 or more total patients were included. The authors found 25 studies including 1555 patients, of whom 696 were evaluable for the effect of *H. pylori* eradication on platelet count. The complete response and overall response (at least doubling of the basal count) were 42.7% (95% CI 31.8–53.9) and 50.3% (95% CI: 41.6–59), respectively. The response rate tended to be higher in countries with a high background prevalence of *H. pylori* infection (e.g., Japan) and in patients with a milder degree of ITP [53].

Suzuki et al. extracted genomic DNA from peripheral blood of *H. pylori*-positive ITP patients, who received eradication treatment, and polymorphisms of IL-1β (−31, −511), IL-1RN (long or short), TNFα (−308), and TNFβ (+252) were analyzed using PCR-restriction fragment length polymorphism. There was no statistical difference in the frequencies of polymorphisms in IL-1β, IL-1RN, and TNFα genes between responders and non-responders. In contrast, the frequency of responders was significantly higher in ITP patients with the TNFβ G/G or G/A genotype than in those with the TNFβ A/A genotype. Therefore, the TNFβ (+252) G/G or G/A genotype may be considered as a good predictor of platelet recovery in ITP patients after *H. pylori* eradication [54]. Asahi et al., in addition to confirming a significant benefit from *H. pylori* eradication therapy in patients with ITP (61 vs 0% among *H. pylori* negative), observed that the recovery in platelet numbers was mediated through a change in FcR balance toward the inhibitory FcγRIIB [55]. The best pathogenic model postulated is based on the antibody cross-reaction between *H. pylori* ureB and human platelet GP IIIa [56].

**Iron-deficiency Anemia (IDA)**

Several seroepidemiologic studies have suggested a link between *H. pylori* infection and IDA both in adults and in children [1,57]. Moreover, pregnant women with IDA had a significantly high prevalence of active *H. pylori* infection [58].

Some investigators observed that cure of the bacterial infection is followed by improvement and normalization of mean cell volume, ferritin, and iron, with disappearance of anemia [59]. During a follow-up of 40 months of children in rural Alaska, *H. pylori* eradication modestly reduced the prevalence of iron deficiency and substantially reduced that of IDA [60]. Different results have been achieved in Iran, where the frequency of *H. pylori* infection in children with and without anemia was similar (44 vs 50%). Among infected children, 36% had anemia vs 42.2% (*p* = .59) in non-infected ones [61]. Similar findings have been reported in Northwest Turkey where authors hypothesized that IDA might be explained by inadequate dietary intake [62]. In Bangladeshi children, the authors observed a significantly higher effect of iron-alone therapy compared to anti-*H. pylori* therapy in improving iron status. Even anti-*H. pylori* treatment compared with placebo was not effective in improving iron status at day 90. No
additional impact of combined anti-\( H. pylori \) plus iron therapy over iron therapy alone was observed [63]. Muhisen & Cohen performed a systematic review and a meta-analysis on \( H. pylori \) infection and iron stores. Although very few studies controlled for multiple potential confounders, most investigations reported a positive association between \( H. pylori \) and decreased body iron stores in symptomatic and asymptomatic infected subjects. \( Helicobacter pylori \) may be considered a risk factor for reduction of body iron stores, iron deficiency and IDA, especially in high-risk groups. The meta-analysis showed an increased risk of IDA (OR: 2.8; 95% CI: 1.9–4.2) as well as iron deficiency (OR: 1.38; 95% CI: 1.16–1.65) [64].

In an elegant study, Lee et al. evaluated the expression of iron-repressible outer membrane proteins (IROMPs) in \( H. pylori \) and its association with IDA. IROMPs were found in IDA strains under iron-restricted conditions. Thus, since specific \( H. pylori \) strains associated with IDA demonstrated an advantage in iron acquisition due to a higher expression of IROMPs, this explains in part why some infected patients are more prone to developing clinical IDA under restricted iron conditions [65].

Monoclonal Gammopathy

Monoclonal gammopathy of unknown significance is not associated with \( H. pylori \) infection. Soler et al. compared the follow-up of 13 patients successfully treated for \( H. pylori \) and 33 who were not cured. After a median follow-up of 19.6 months, the monoclonal component was unchanged in eradicated, and not different between eradicated patients compared to those with \( H. pylori \) negativity ab initio (15.6 ± 9 vs 15.9 ± 1 vs 15.7 ± 1) [66].

Hepatobiliary Diseases

There is an increasing interest in \( Helicobacter \) species’ role in human liver diseases, even though results are still inconclusive and supported by only a few papers, mainly based on animal models [67]. Ito et al. by using transmission electron microscopy, found in vitro differences between hepatocytes and gastric epithelial cells in terms of both adherence and internalization of \( H. pylori \). Of interest is the fact that the bacterium adhered and was internalized into hepatocytes more efficiently than into gastric epithelial cells \((p < .05)\). Once inside the hepatocytes, both VacA-positive and -negative \( H. pylori \) strains were able to produce vacuoles, interpreted as endocytotic vesicles. \( \beta 1 \)-integrin was identified as a probable receptor involved in internalization of \( H. pylori \) into hepatocytes [68]. The same group observed that \( H. pylori \) infection of hepatocytes causes disturbance of apoptosis and DNA synthesis. In particular, infection with more virulent strains resulted in cell arrest and increased DNA fragmentation. The difference with the less virulent strain employed (\( H. pylori \) 401C) was significant [69]. Goo et al. induced lesions resembling those of human primary biliary cirrhosis (PBC) in a 24-month-old male C57BL/6 mouse infected with \( H. pylori \). Since the serum antivacuolating toxin IgG in this mouse showed the highest value in the \( H. pylori \)-infected group, the authors concluded that the increase in vacuolating toxin caused by \( H. pylori \) infection may be related to the development of PBC by molecular mimicry [70].

Finally, an in vitro study showed that \( H. pylori \) significantly influenced human gallbladder epithelial cell morphology, causing reduced cell growth, decreased viability, and increased detachment. The damage was more significant in cells treated in culture liquid/broth than in \( H. pylori \) sonicate extracts [71].

Diseases of the Large Intestine

The potential association between \( H. pylori \) infection and colorectal diseases has been investigated in the last year.

In the human colon, \( Helicobacter \) sp. DNA can be found in 35% of cases [72]. However, its pathogenic role, if any, remains to be elucidated.

Regarding the field of oncology, Zhao et al. performed a meta-analysis on \( H. pylori \) and the risk of colorectal cancer, including 13 studies. The summary OR was 1.49 (95% CI: 1.17–1.91). Moreover, with the method of fail-safe, the effect of publication bias was small [73]. In a large study, including 685 subjects, the authors found that neither hypergastrinemia nor serologic evidence of \( H. pylori \) infection was associated with an increased risk of recurrent adenoma development [74]. Soylu et al. looked for the presence of \( H. pylori \) by immunohistochemistry in samples of patients undergoing polypectomy during colonoscopy. They found that 21.6% of all specimens were positive, with a percentage of 25 in the case of cancer. The presence of \( H. pylori \) in colon polyps did not yield any correlation with polyp size, colonic localization or histopathologic type [75].

Gynecological Diseases and Fertility Disorders

Nausea and vomiting are very frequent complaints of pregnant women and, when severe, may lead to hyperemesis gravidarum (HG), characterized by weight
loss, dehydration, acidosis from starvation, alkalosis from loss of hydrochloric acid in vomitus, hypokalemia, and transient hepatic dysfunction. While some seroepidemiologic studies showed that H. pylori infection is significantly associated with HG, others did not [1]. Sandven et al. included in a case-control study, 244 women with HG and 244 pregnant women free of HG. They observed that the presence of H. pylori increased the risk of HG by more than twofold (OR: 2.42, 95% CI 1.64–3.57, \( p < .001 \)). The association was significantly more evident among Africans than in non-Africans [76]. Pugliese et al., comparing 25 pre-eclamptic women and 25 healthy parturient, have shown that the former had a significantly higher H. pylori seropositivity as well as a higher anti-CagA seropositivity. On the contrary, they did not find a difference in IL-18 levels between the two groups [77]. The benefit of H. pylori eradication in patients with localized vulvodynia, reported in a study with a small sample size, remains to be better understood [78].

Regarding infertility, Collodel et al. showed that infertile patients infected with H. pylori had a low sperm quality compared to uninfected ones. A significant reduction of sperm motility and fertility was observed, particularly in CagA-positive patients, whereas apoptosis and necrosis were increased (\( p < .05 \)). In these, the mean values of TNFα levels were higher than those of uninfected patients. The percentage of immaturity and the related defective organelles did not seem to be influenced by the presence of the bacterium [79]. Based on a study in which both the anti-H. pylori antibodies in serum and follicular fluids were measured, Kurotsuchi et al. hypothesized the presence of an antigenic mimicry between the flagella of H. pylori and spermatozoa. Thus, antibodies produced against H. pylori flagella may cross-react with spermatozoid flagella, increasing the risk of infertility [80].

**Neurologic and Psychiatric Diseases**

In this field, in the last year more hypotheses and reviews than original data have been published [81,82]. Among the latter, Kountouras et al. investigated the benefit of H. pylori eradication in the management of Alzheimer’s disease. They showed that the prevalence of the bacterial infection was significantly higher in patients than in age-matched controls (88 vs 46.7%, \( p < .001 \)). Helicobacter pylori eradication was obtained in 84.8% of the cases. After a 2-year follow-up, patients who were cured of the infection had a significant improvement in cognitive and functional status parameters (measured by specific scales) compared to those who failed eradication [83]. Although it is difficult to come to a definite conclusion on the causality of the relationship between H. pylori and Alzheimer’s disease, and the consistency of an association based on published studies is poor [84], this prospective study on a small sample size provides the basis for well-designed trials with larger populations.

Regarding Parkinson’s disease, Lee et al. showed that infected patients had a longer L-dopa “onset” time and a shorter “on-time” duration than uninfected patients (\( p < .05 \)). Helicobacter pylori eradication improved the delay in the L-dopa “onset” time and the short “on-time” duration. According to the authors, these data demonstrate that the bacterium could interfere with the absorption of L-dopa and provoke motor fluctuations [85].

In a study on sera from 120 individuals affected by neuropsychiatric lupus compared to those from 140 geographic controls, Zandman-Goddard et al. failed to find an association between this disease and seropositivity for H. pylori antibodies [86].

**Other Diseases**

Confirming a previous study by Demir et al. [87], a seroepidemiological investigation conducted on a random sample of patients obtained from the previously cited MESA study, did not show an association between H. pylori infection and type 2 diabetes mellitus [88]. On the contrary, in South America, Fernandini-Paredes et al. found that in diabetic patients, glycosylated hemoglobin levels were higher in infected than in uninfected individuals (\( p = .03 \)). The presence of the bacterium was not associated with the response to the diabetes mellitus treatment [89]. On a similar trend, another group showed that H. pylori infected patients had a significantly higher HOMA-IR level (diagnostic standard of insulin resistance) when compared with uninfected ones [90]. In a Japanese case report, the authors reported the onset of type 1 diabetes mellitus after H. pylori eradication [91]. The link with an autoimmune mechanism remains unclear.

The circulating levels of ghrelin and leptin, two hormones involved in body weight regulation and food intake, in relation to H. pylori status in adult males have been investigated. Chuang et al. observed that, before H. pylori eradication, males had lower plasma ghrelin levels than females (\( p < .001 \)), but thereafter these levels were similar. Such a gender difference was not evident for leptin levels [92]. Based on previous work leading to the hypothesis that gastric H. pylori colonization reduced circulating levels of leptin and ghrelin, Roper et al. examined gastric, circulating, and gastric juice levels of leptin and ghrelin in fasting
**Helicobacters and Extragastric Diseases**

_H. pylori_-positive and _-negative adult male subjects. They showed that bacterial colonization was associated with reduced circulating leptin levels, independent of body mass index, and fundic ghrelin and leptin levels were directly related. Levels of ghrelin in infected and uninfected patients were similar. Ghrelin was present in gastric juice over a large concentration range, and was strongly correlated with gastric pH [93]. Gao et al. observed that ghrelin concentration and ghrelin/obestatin ratios were lower in _H. pylori_ infected subjects than in uninfected individuals [94]. Obestatin is a peptide derived from preproghrelin and a physiologic opponent to ghrelin. Interestingly, a group found that autoantibodies against appetite-regulating peptide hormones and neuropeptides were displayed by both healthy humans and rats. The authors hypothesized a potential link between gut microflora and appetite control [95].

_Helicobacter pylori_ infection also seems to play a role in conjunctival MALT lymphoma, for which a mechanism similar to those of gastric MALT lymphoma has been postulated [96], but not in glaucoma [97]. However, in the latter, Deshpande et al. found a higher seroprevalence of _H. pylori_ IgG antibodies in patients with primary open angle glaucoma compared with those with pseudo-exfoliation glaucoma. This difference was not evident upon analysis of the aequous humor [98]. Choi et al. investigated the association between _H. pylori_ infection and Posner–Schlossman syndrome and found that patients had a significantly higher seroprevalence than controls (80 vs 56.2%, _p_ = .014) [99].

As reported in the section on respiratory diseases, the relationship between _H. pylori_ infection and allergic diseases is of increasing interest. All studies converge towards a lack of positive association. In a large cohort of 1953 Japanese university students, allergic diseases were frequent and negatively associated with _H. pylori_ infection, especially in men [100]. This is in agreement with other data [101,102]. Cam et al. observed a counteractive Th1 and Th2 cytokine interaction between _H. pylori_ infection and atrophy [103]. This effect was not protective, as opposed to conclusions made in a study by Konturek et al., in which _H. pylori_ infection was associated with a decreased risk of food allergy [104].

Two nested case–control studies, including 104 [105] and 87 [106] patients with pancreatic cancer, concluded that _H. pylori_ infection was not associated with the development of this neoplasm. However, in the latter investigation, an association was found in subjects who were not smokers (OR: 3.81; 95% CI: 1.06–13.63) [106].

With regard to dermatology, two intervention studies have reported opposing results: _H. pylori_ infection was not associated with chronic urticaria in Germany [107] to the contrary of India, where it has been suggested to include the detection of this bacterium in the diagnostic work-up of this disease [108]. The data by Abdel-Hafez et al. supported the hypothesis that _H. pylori_ infection is not associated with alopecia areata [109]. The clearance of chronic psoriasis after eradication therapy for _H. pylori_ infection has been the focus of a case report [110], and a case report of _Helicobacter cinaedi_ bacteremia in a previously healthy person with cellulitis has also been described [111].

Ozel et al. observed that _H. pylori_ eradication in patients with familial Mediterranean fever led to a decreased level of IL-6. These findings were not evident in subjects homozygous for M694V mutation [112].

One case report has shown that anti- _H. pylori_ treatment can reduce, for a limited time, the recurrence of oral aphthous ulcers in patients with Behcet’s syndrome [113]. It is important to understand if this temporary benefit is due to the antibiotics or to _H. pylori_ eradication. A study on 23 patients with recurrent aphthous stomatitis showed that, after bacterial eradication, there was a significant reduction of the recurrence and amelioration time [114].

In a study on patients with sudden infant death syndrome, a significant prevalence of active _H. pylori_ infection (by stool antigen) among infant death cases compared with live controls was observed [115].

**Conflicts of Interest**

The authors have not declared any conflicts of interest.

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**Helicobacter spp. Other Than Helicobacter pylori**

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**Detection Methods, New Species and Prevalence in Animal Hosts**

Molecular biology techniques provide invaluable tools for the identification of Helicobacter spp., and the gold standards in the detection of this genus are PCR and/or sequencing. Evaluation of 16S rDNA gene-based PCR assays for genus-level identification of Helicobacter spp. in fecal samples demonstrated that five of the six methods examined were appropriate to eliminate PCR inhibitors from the samples [1]. The method recommended in this work was the application of PCR-differential gradient gel electrophoresis to fecal samples reported previously [2]. A PCR assay performed at the low annealing temperature of 50 °C was developed to amplify a 261-bp sequence spanning two of the seven variable regions in the 16S rDNA gene of Helicobacter species [3]. This assay combined with that of Bohr et al. [4] and sequencing served to detect the presence of Helicobacters in commercially bred mice supposedly free of Helicobacter infection and to identify different species of Helicobacter and their relative proportions infecting a single animal.

A real-time PCR assay that amplifies a fragment from the 16S rDNA gene with subsequent species identification by melting curve analysis using SYBR Green chemistry was designed to detect Helicobacter pullorum-like organisms in chicken products [5]. The method allows identification of these bacteria from healthy poultry carcasses and caeca with a sensitivity of 1 CFU/g. It confirmed the inadequacy of culture methods in the detection of H. pullorum-like bacteria, and showed that these organisms are common in healthy chickens with a prevalence similar to that of Campylobacter jejuni [6]. As H. pullorum is considered to be zoonotic [7], this PCR assay will serve to better assess the potential human health risks posed by this bacterium.

**Keywords**

Enterohepatic Helicobacter spp., new Helicobacter spp., detection of infection, animal studies, prevention, eradication.

**Abstract**

Non-*. pylori* Helicobacter species (NPHS) are associated with several important human and animal diseases. In the past year research into this group of bacteria has continued to gain attention, and novel species have been described in new niches owing to improvements in detection methods. Polymerase chain reaction and/or sequencing remain the gold standard for the detection of this genus. New insights into the pathogenesis of the NPHS in hepatobiliary, gastric, and intestinal diseases were gained. In particular, data revealed interaction between hepatic steatosis and infectious hepatitis in the development of hepatocellular carcinoma. Evidence of an association between hepatitis C virus and Helicobacter spp. in hepatocarcinoma development was also provided; and male sex hormone signaling appeared to influence infectious hepatitis induced by Helicobacter hepaticus. More findings support an association between Helicobacter heilmannii and gastric adenocarcinoma; and in mice, mucins MUC4 and MUC5 but not MUC1 influence the colonization and pathogenesis of Helicobacter felis. Data indicated that the roles of the adaptive immune system in H. hepaticus-induced intestinal tumorigenesis are different in the small and large intestines, and environmental factors, such as bile acids may modulate H. hepaticus carcinogenic potential. New reports in the prevention and eradication of NPHS showed a protective response against Helicobacter suis induced by vaccine administration, and a successful cross-foster rederivation method successfully eradicated Helicobacter spp. from contaminated mice litters. Overall, the studies provided insights into the pathophysiology of Helicobacter species other than Helicobacter pylori.

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Identification based on the 16S rDNA gene remains the most frequently used method of detection; however, this gene may be a poor target owing to horizontal transfer of 16S rDNA gene fragments and the creation of mosaic molecules with loss of phylogenetic information [8]. Other phylogenetically informative genes such as the 23S rDNA could be employed for species detection, but lack of sequence information limits their potential use as targets. Indeed, among the enterohpatic Helicobacters only the genome of Helicobacter hepaticus has been fully sequenced and annotated. This situation improved recently when the Broad Institute Genome Sequence Platform performed the sequencing of the genomes of Helicobacter bilis ATCC 43879, Helicobacter canadensis MIT 98-5491, Helicobacter cinaedi CCUG 18818, H. pullorum MIT 98-5489, and Helicobacter winghamensis ATCC BAA-430. The final assembly and annotation of these genomes has not been finalized, but the non-assembled contigs are available in the NCBI microbial genomes database.

Helicobacter suis has been accepted as a new gastric Helicobacter taxon corresponding to type 1 H. helimannii [9]. The cells of the new species are tightly coiled spirals with up to six turns, are motile and have bipolar tufts of 4–10 sheathed flagella blunt ended or ending in spherical knobs [9]. An analysis of current data on the prevalence of different species of non-H. pylori gastric Helicobacter spp. in humans concluded that H. suis is a zoonotic agent [10].

During the past year active investigations were conducted on the presence of Helicobacter spp. in wild, husbandry and pet animals, and the zoonotic potential of Helicobacter spp. was evaluated owing to the potential transmission of these bacteria from animals to humans.

Evidence for transmission of Helicobacter spp. in the marine environment was obtained from pools of captive mammals through PCR amplification and sequencing of DNA. The gastric Helicobacter spp. detected were homologous to Helicobacter cetorum, and the enterohpatic Helicobacter spp. were homologous to that isolated from a Northern sea lion [11]. Helicobacter DNA with very high homology to H. cetorum was detected in South American fur seals indicating a wide host range for this Helicobacter species initially isolated from whales and dolphins [12].

Helicobacter DNA was detected by PCR in river water but not in soil in Sapporo, Japan, and its presence was not related to that of Acanthamoeba, initially suspected to be involved in Helicobacter survival [13]. A study of the survival in water of seven gastric and enterohpatic Helicobacter spp. did not show relationship between survival time in water and the niche occupied in the host, and concluded that water would have similar roles in the transmission of these species [14].

Several studies provided new information on Helicobacter colonization of mammals. For the first time Helicobacter 16S rDNA was detected in the stomach of lynx and foxes. Phylogenetic analyses grouped the isolates in a cluster of H. helimannii, Helicobacter salomonis, H. felis, and Helicobacter bizzozeronii [15].

The prevalence of the new species Helicobacter equorum in foals is age dependent, and the differences in prevalence may be related to the presence of protective maternal immunity in the very young foals [16].

No significant relationship was found in pet dogs between chronic gastritis and Helicobacter infection [17], and none of the common culturable species found in the stomach of dogs including H. salomonis, H. felis, H. bizzozeronii, and Helicobacter rappini was identified [17]. Genus-specific Helicobacter-positive samples were found in the saliva or feces of domestic and feral cats in Korea; all samples were negative for the detection of H. felis and H. pylori with species-specific probes [18].

Mixed infections of bacteria of the genera Campylobacter, Helicobacter, and Anaerobiospirillum were found in cats and dogs, but no significant statistical correlation was found between the presence of diarrhea in either cats or dogs and any isolate of the three genera, or the various co-infection rates [19].

Helicobacter spp. detected by PCR amplification of 16S rDNA sequences in gastric washings of cats showed high homology with those of H. helimannii and Helicobacter acynonychis [20]. The data suggest that the proposed method was a valuable alternative technique to gastric biopsy. Since virtually all healthy adult cats harbor Helicobacters in their gastric mucosa, the possibility of cats as potential zoonotic agents of H. helimannii may have important public health implications [20].

A review on the Helicobacter spp. infections of domestic cats and dogs, farm animals, birds, and several wild animals concluded that a common pathogenic mechanism is the induction of a Th1-driven chronic inflammatory response mounted by the host against the bacteria [21].

Diseases

Hepatobiliary Diseases

In the past year, the potential association between Helicobacter spp. and diseases of the hepatobiliary tract continued to gain attention. Exposing the mouse hepatocyte cell line H2.35 to H. pullorum sonicates resulted in the hepatic cell death with morphological features of necrosis, which occurred without caspase-3 activation [22]. The necrosis and eventual cell death
was attributed to features characteristic of mitotic catastrophe, such as chromatin condensation, formation of multinuclear distended cell micronucleation, and intranuclear pseudoinclusions. The toxic factor in the sonicates had similar properties to those of the cytolethal distending toxin secreted by *H. pullorum* [23,24].

The significance of the route of infection in *H. hepaticus*-induced hepatitis on the development of hepatocellular carcinoma (HCC) was evaluated in a mouse model by testing the hypothesis that perinatal exposure to *H. hepaticus* is required for liver tumorigenesis [25]. Male A/JCr mice infected with *H. hepaticus* by intragastric treatment developed early hepatic changes after exposure as well as marked increase in oxidative DNA damage, but rarely any liver tumor, confirming earlier reports [26,27]. In contrast, infection of A/JCr mice by intraperitoneal injection of *H. hepaticus* before breeding led to a high incidence of progressive hepatitis and a significant number of multiple liver tumors, including HCC, in the male offspring [25]. Contributing perinatal factors include high sensitivity of neonatal liver to tumor initiation, and/or modulation of immune response by *H. hepaticus* or its toxins [25].

The oncogenic potential of *H. hepaticus* was further illustrated using the susceptible mouse strains AB6F1 and B6AF1 derived from A/JCr and C57BL/6NCr mice, respectively [28]. The results suggested synergistic interactions between hepatic steatosis and infectious hepatitis leading to HCC. The study provides an alternative novel mouse model to investigate the association between chronic microbial hepatitis and fatty liver in the pathogenesis of liver cancer.

The influence of male sex hormone signaling on infectious hepatitis induced by *H. hepaticus* was studied in A/JCr mice [29]. At 4 months, castrated males and animals receiving the competitive androgen receptor antagonist flutamide had significantly less severe hepatitis than intact controls. The results raised the possibility of targeted hormonal therapy in young male patients with infectious HCC.

In patients suffering from cholangiocarcinoma in Thailand, a high cross reactivity was found between the immune response to antigens obtained from *H. pylori* and *H. bilis* to the extent that infection with the two species cannot be distinguished serologically in most subjects given the high prevalence of *H. pylori* in the population studied [30]. Retrospective analyses of serum antibody responses to cell surface proteins of *H. pullorum*, *H. bilis*, *H. hepaticus*, and *H. pylori* suggested an association between hepatitis C virus and the development of HCC [31]. In contrast, no association was found between *Helicobacter* spp. and biliary tract cancers [32].

**Gastric Diseases**

Several studies on animals reported an association between NHPHS and gastric diseases. *Helicobacter heilmannii* induced low-grade mucosa-associated lymphoid tissue-type (MALT) lymphoma in mice increasing the microcirculatory network surrounding the lymphoma tissue [33]. In pet cats, *H. heilmannii* infection was associated with the development of gastritis and feline MALT lymphoma [34]. In Iranian pet dogs, 93% of gastric samples from animals with chronic gastritis or histopathological changes in the gastric mucosa showed the presence of *Helicobacter* spp. DNA [17].

Long-term *H. heilmannii* infection causes Th1 and Th2 immune response and increases mucosal thickness of the stomach of C57BL/6 mice [35]. Interestingly, co-infection with *H. bilis* and *H. pylori* in this mouse breed induces less severe gastritis, atrophy, mucous metaplasia and hyperplasia, as well as less severe intestinal metaplasia and dysplasia than *H. pylori* infection alone [36]. The explanation suggested for the reduced pathology is the migration of *H. bilis*-primed immune regulatory cells in the lower bowel to the gastric compartment and downregulation of the Th1 response.

The inflammation induced by *H. pullorum* in human gastric and intestinal epithelial cell lines occurs via bacterial adherence probably through lipopolysaccharide-induced IL-8 secretion and is mediated by NF-κB signaling [37].

*Helicobacter felis* attachment to gastric epithelial cells in vitro was limited by the constitutively expressed glycoprotein of gastric epithelium mucin MUC1 [38]. However, this mucin did not impact on the bacterial in vivo colonization or pathogenesis in contrast to previous observations with *H. pylori*. The results provide evidence that *H. felis* colonizes and elicits inflammation in vivo without direct association with the gastric mucosa, and that in mice MUC1 shed by epithelial cells does not play an important role in limiting colonization or pathogenesis of these non-adherent bacteria. The data suggest that inflammation induced by *H. felis* infection results from shed antigens which act independently of MUC1 [38].

Alterations in gastric mucins and trefoil factors expression were investigated in two C57BL/6 mouse strains infected with *H. felis* as the disease provoked by the presence of the bacterium progresses from gastritis through dysplasia and metaplasia to gastric carcinoma. The expression of mucins MUC4 and MUC5b increased, and that of MUC5ac decreased; these changes were similar to those found in the expression of human mucins in *H. pylori* infection and the data suggested a role for MUC4 and MUC5b in disease progression in mice.
At variance with the observations in human disease, other murine mucins and trefoil factors remained unchanged [39]. Earlier reports of an association between H. helman-nii infection with gastric adenocarcinoma in humans [40] were supported by the finding that biomarkers of gastrointestinal cancers were elevated in patients suffering from gastric ulcer and cancer caused by H. helman-nii infection [41].

**Intestinal Diseases**

New insights have been obtained on the pathogenicity of enterohepatic *Helicobacter* spp. employing mouse animal models. Co-infection of IL-10-deficient mice with *Helicobacter rodentium* and *Helicobacter typhlonius* resulted in more severe inflammatory bowel disease and neoplasia compared to the disease caused by either *H. rodentium* or *H. typhlonius* [42]. The anti-inflammatory effects of an antibiotic quadruple therapy in Helicobacter-infected and non-infected IL-10−/− mice with colitis suggested that together with *Helicobacter* spp. other microbea drive the inflammatory process in these mice [42].

The urease activity of *H. hepaticus* is not involved in cecal colonization of A/JCr male mice but is essential for hepatic colonization and plays a crucial role in liver inflammation and the severity of hepatitis [43]. Urease activity was also associated with higher total IgG, Th1-associated IgG2a and Th2-associated IgG1 in vivo. The sequences of *H. hepaticus* UreA and UreB are very similar to those of *H. pylori*, but these proteins are not as immunodominant as the *H. pylori* ones. The proline utilization A (PutA) flavoenzyme of *H. hepaticus* is not involved in the colonization efficiency of mice but rather in inflammation, suggesting a role for proline metabolism in *H. hepaticus* pathogenicity [44].

Studies of infection of BALB-Min and BALB-RagMin mice with *H. hepaticus* indicated that the bacterium promotes colon but not small intestine tumorigenesis, and suggested that *H. hepaticus* promotion of tumors differs between organs and does not necessarily correlate with severity of inflammation [45]. The data also indicate that the roles of the adaptive immune system in tumor-igenesis are different in the small and large intestines. Environmental factors, such as bile acids present in the intestinal lumen, may modulate *H. hepaticus* carcinogenic potential.

Induction of colon cancer by *H. hepaticus* infection in recombinase-activating gene-2-deficient *Rag2−/−* mice is mediated by inflammation, increased TNF-α and nitric oxide production (NO) production [46]. Concurrent administration of an inducible nitric oxide synthase inhibitor prevents NO production, abrogates epithelial pathology and inhibits the onset of cancer [46].

The pathogenic potential of the *H. hepaticus* genomic island HHGI1 was investigated in IL-10−/− mice employing the isogenic bacterial mutant HhPAId1 that lacks 19 predicted genes within HHGI1. *Helicobacter hepaticus* HhPAId1 did not cause typhlocolitis and hyperplasia in IL-10−/− mice [47]. Colonization levels of HhPAId1 were significantly higher in the cecum and similar in the colon compared to wild-type *H. hepaticus*. The results suggested that genes in HHGI1 contribute to the pathogenicity of *H. hepaticus*, at least in part via upregulation of proinflammatory mediators IFN-γ, TNF-α, and IL-17a [47].

Testing of the hypothesis that prior infection of BALB/c-IL-10 null mouse with *H. hepaticus* increases the incidence, multiplicity and/or progression of either colitis-associated adenocarcinomas or colon tumors induced by the organotrophic carcinogen azoxymethane (AOM) showed that prior infection with *H. hepaticus* had no effect on the incidence of colitis-associated adenocarcinomas, and resulted in a significant increase in incidence but not multiplicity or progression, of AOM-induced polypoid tumors [48]. On the other hand, Munday et al. [48] found no association between *Helicobacter* spp. infection and ovine small intestinal adenocarcinomas in New Zealand sheep [49].

Enterohepatic and gastric *Helicobacter* spp. were identified in fecal specimens from children diagnosed with Crohn’s disease using PCR. The data suggest that in a considerable proportion of children *Helicobacter* spp. may have a pathogenic role in the development of the disease [50].

The effects of Helicobacter infection on the toxicity of the contaminant 2,3,7,8-tetrachloro-dibenzo-p-dioxin was investigated in rats. The infection appeared to have little influence on the susceptibility of rats for the compound [51].

**Prevention and Eradication of Infection**

The effects of prophylactic immunization of BALB/c mice against *H. suis* using whole cell lysate or supernatant antigens from in vitro cultured bacteria showed that a protective response against the bacterium can be induced by administering a vaccine by intranasal route with homologous (*H. suis*) as well as heterologous (*H. bizzozeronii* and Helicobacter cynogatricus) antigens [52].

A successful cross-foster redervative method was devised for the elimination of *Helicobacter* spp. from contaminated mice litters [53].

Triple therapies using amoxicillin and two other active components showed high efficacy against gastric Helicobacter infections in C57BL/6J mice [54] and cats and dogs [55]. Amoxicillin resistance in *H. hepaticus* is
dependent on the hflA gene expressing a TolC component of a putative efflux system and its expression is induced by bile acids [56]. The role of a second urease expressed by the gastric Helicobacter acinonychis, H. felis, and Helicobacter mustelae which does not require activation by accessory proteins or nickel was proposed as an adaptation to the nickel-restricted diet of carnivores [57].

Conclusions

This past year saw continued interest in the study of Helicobacter species other than H. pylori that resulted in significant amount of information on improvement of detection methods for the genus, identification of novel species and prevalence in animal hosts, pathogenic mechanisms of disease causation, prevention, and eradication of infection. Understandably, a greater proportion of the investigations focused on the relationship between Helicobacter spp. and diseases of the hepatobiliary and gastrointestinal tracts, and provided new insights into the potential mechanisms that buttress the association of these bacteria with liver, stomach, and intestinal cancer. Continued study in this area is required given the global need to eradicate these malignancies.

Conflict of Interest

The authors have declared no conflicts of interest.

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