

# *Helicobacter pylori*: 20 years on

Barry Marshall

**ABSTRACT** – *Helicobacters* are a new genus of bacteria, inhabiting the interface between mucosa and lumen of the gut. Microaerophilic, spiral, flagellated and urease positive, they possess features necessary for colonisation of the juxtamucosal mucus environment. *Helicobacter pylori* is the major pathogenic species. Once attached to the gastric epithelial cells, it incites an immune response characterised histologically by the development of active gastritis and immunologically by the presence of specific IgG. Persistence of infection is ensured by attachment to tissue antigens (eg Lewis B), a vacuolating toxin (VacA) which assists the free passage of urea through epithelial cells, and a cytotoxin (CagA) which is actually injected into the epithelial cells via a Type IV secretion system. Finally, during the typical lifelong chronic infection, two important diseases occur. *H. pylori* alters gastric physiology to cause acid hypersecretion and peptic ulcer. Secondly, it damages the acid secreting mucosa leading to atrophic gastritis and gastric cancer risk.

**Key words:** *cagA*, gastritis, helicobacter, peptic ulcer, stomach cancer, toxin, ulcer, urease, VacA

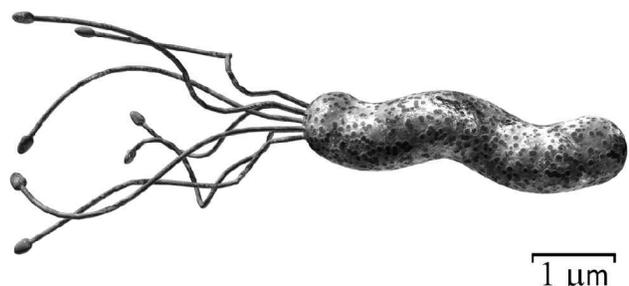
This year marks the 20th anniversary of the culture of *Helicobacter pylori* in Perth, Australia, over Easter 1982. It is now known that the spiral, Gram-negative bacterium inhabits the stomach of more than 50% of humans. Although the new organism was only cultured in 1982<sup>1</sup>, the Italian anatomist Giulio Bizzozero had reported its manifestations in the scientific literature 100 years earlier<sup>2</sup>. He recognised that dogs harboured a gastric ‘spirochaete’ – certain proof that bacteria could survive in the acid-secreting stomach. Other investigators noticed that urease was usually present in the stomach of carnivorous animals such as dogs and cats<sup>3</sup>, findings that were extended to humans in 1950<sup>4</sup>. It was thought that all these facts were unrelated, and that gastric urease was actually secreted by the gastric epithelial cells, until Charles Lieber showed that it could be suppressed with tetracycline<sup>5</sup>, and Delluva showed that germ-free animals did not develop gastric urease<sup>6</sup>. The presence of spiral bacteria in the human stomach was reported several times, most notably by Freedberg in 1940<sup>7</sup> and Steer and Colin-Jones in 1975<sup>8</sup>, well before the

bacteria were found to cause peptic ulcer and gastric cancer<sup>1,9</sup>. The history of *H. pylori* is the subject of a recent book, *Helicobacter pioneers: firsthand accounts from the scientists who discovered helicobacters, 1892–1982*<sup>10</sup>.

## Microbiology and taxonomy

*H. pylori* is named because of its spiral or helical shape (Fig 1). The organism is approximately 0.6 µm thick, taking the shape of a flat spiral with 1.5 wavelengths. The organism has up to seven sheathed flagella. It prefers a microaerophilic (reduced oxygen) environment and can be cultured in gas jars with *Campylobacter* gas generating envelopes (Oxoid), or in carbon dioxide (CO<sub>2</sub>) incubators at 37°C. The preferred agar media contain blood, with or without selective antibiotics, but media without blood can also be used<sup>11</sup>. The first helicobacter cultured was actually *H. muridarum*, a commensal organism colonising the crypts of the mouse caecum<sup>12</sup>. New helicobacter species are discovered regularly, and some are now regarded as human pathogens. Figure 2 summarises the taxonomy of the helicobacter genus as determined by sequencing the 16s ribosomal RNA<sup>13</sup>.

According to observations from human and animal helicobacter infections, the genus has the capacity to colonise and cause inflammation in the stomach (*H. pylori*, *H. heilmannii*, *H. mustelae*), colon (*H. fennelliae*), and, in mice, liver (*H. bilis*, *H. hepaticus*). *H. pylori* colonises the mucus layer which lines the human stomach, and its relatives occupy similar ecological niches in the gastrointestinal tracts of animals.



**Fig 1.** *Helicobacter pylori* 3-D image. Reproduced with permission from Concrete Bob Software.

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*Clin Med JRCPL* 2002;2:147–52

Genomes of at least two isolates of *H. pylori* have been completely sequenced<sup>14,15</sup>.

## Epidemiology and disease associations

Although so many people are infected, the exact mode of transmission of *H. pylori* is the source of some controversy. In developing countries, usually 50–90% of the population is infected with the organism, and children acquire the infection soon after being weaned. For example, in West Africa, 80% of children may be infected with the organism by age five. The overriding association with *H. pylori* is lower socio-economic status during childhood. This explains the high prevalence of the organism in developing countries, and also the increased presence of the bacterium in adults who live in crowded, unsanitary conditions (small houses, sharing a bed with siblings, absence of hot running water) even in developed countries<sup>16</sup>. In developed countries, *H. pylori* does not seem to be acquired from the environment, and persons below the age of 40 years are usually uninfected or have rather low prevalence of the organism (5–20%). In countries whose economy has gone from developing to affluent over the past 50 years, marked decline in *H.*

*pylori* prevalence has been noted. The best example of this may be Japan. More than 50% of adults over the age of 40 are infected, reflecting poor conditions in that country before 1960 when they were children. Since 1970, however, new infection with *H. pylori* has been far less common, and today less than 5% of Japanese children acquire the infection<sup>17</sup>.

Transmission of *H. pylori* is probably by the faecal-oral route since the organism has been detected in, and associated with, contaminated water, most notably in Peru<sup>18</sup>. *H. pylori* survives for a time in gastric juice. All of us reflux slightly from time to time, so it is not surprising that the organism, and more commonly its DNA, has been detected in the mouth, for instance in dental plaque<sup>19,20</sup>. Recent observations in volunteers infected with *H. pylori* who were made to vomit or have diarrhoea showed that an actively unwell person with these symptoms could spread *H. pylori* in the immediate vicinity by aerosol, splashing of vomitus, infected vomitus and infected diarrhoea<sup>21</sup>.

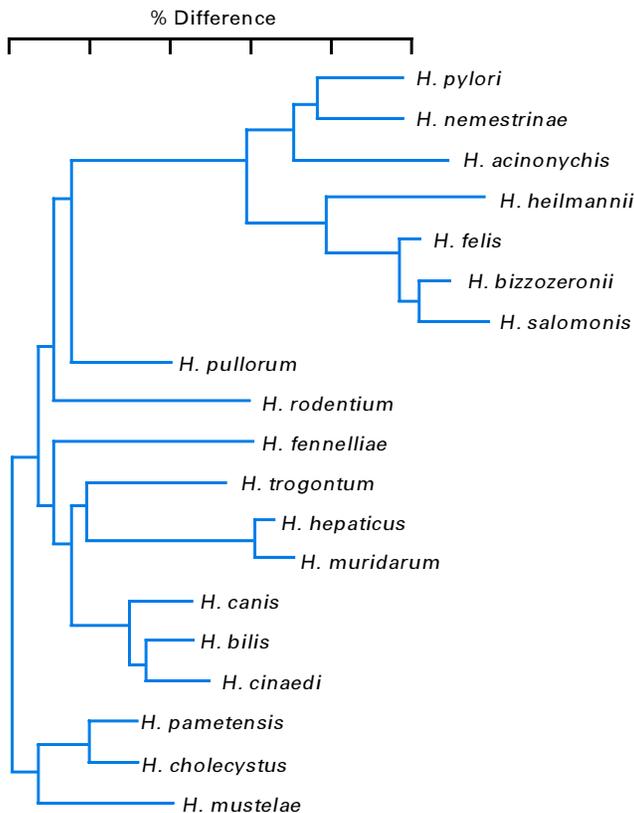
In summary, *H. pylori* is usually spread by the faecal-oral route, but possibly also by the oral-oral route and the spread of contaminated secretions. Thus, in developing countries, individuals catch *H. pylori* at a very young age from other persons (children) in their environment. In developed countries, *H. pylori* is more difficult to acquire, and is usually transmitted from one family member to another, most likely by the faecal-oral route, but possibly also by the oral-oral route (eg kissing, vomitus, etc). The presence of many small children in the family appears to increase the infectivity of the infection.

## Ulcers and stomach cancer

Diseases commonly associated with *H. pylori* are shown in Fig 3. Assuming that about 30% of people are infected with *H. pylori* (hatched circle), most of the gastric diseases (duodenal ulcer, gastric ulcer and gastric cancer) occur in those with the infection. Notably, the most common lymphoma of the stomach, mucosa associated lymphoid tissue (MALT) lymphoma, is strongly associated with *H. pylori* infection and usually goes into complete remission when the infection is eradicated<sup>22</sup>. Numerically, gastric cancer is the first or second most common cancer in many developing countries, where incidence may be 40–60 per 100,000 population per annum. In developed countries, gastric cancer is less important, but affects 5–10 per 100,000 population per annum, which in the USA translates into several thousand deaths annually.

By causing chronic inflammation of the gastric mucosa and subsequent alteration of the mucosa from gastric mucus-secreting epithelial type II (intestinal metaplasia), *H. pylori* predisposes the sufferer to gastric cancer. Paradoxes exist in the epidemiology, however, because gastric cancer is rather uncommon in parts of Africa where *H. pylori* is prevalent (the 'African Enigma'<sup>23</sup>). In most countries, *H. pylori* infection is associated with a 4–6 fold increased risk of gastric cancer. This means that more than half the gastric adenocarcinomas in the world are related to *H. pylori* infection.

Two recent developments have reinforced this concept. First, Japanese investigators observed that infection of Mongolian



**Fig 2. Taxonomy of *Helicobacter* species by 16s RNA.** There are many species of *Helicobacter*. This dendrogram shows the classification of *Helicobacters* based on 16s RNA sequencing. Notice *Helicobacter pylori* at the top of the chart and the closely related *H. nemestrinae* (*Helicobacter* from macaques). Classification is: Bacteria \_ Proteobacteria \_ epsilon subdivision \_ *Helicobacter* group \_ *Helicobacter* \_ *Helicobacter pylori*.

gerbils with *H. pylori* led to the development of gastric ulcer six months later and then to gastric adenocarcinoma after 12 months<sup>24</sup>. Secondly, in a preliminary study from Hiroshima, eradication of *H. pylori* from individuals in whom small *in situ* gastric carcinomas had been resected resulted in less recurrence of gastric carcinoma compared with follow-up studies with individuals in whom *H. pylori* infection was allowed to continue<sup>25</sup>. Asian physicians are optimistic that treatment of *H. pylori* in persons at high risk for gastric cancer, together with the gradual reduction of *H. pylori* in the whole population since about 1970, will result in far fewer cases of gastric cancer in the future. Strong evidence that this improvement is already underway was recently shown by Uemura<sup>26</sup> in a prospective Japanese study. Gastric cancers did not develop in 280 individuals who were free of *H. pylori*, whereas 36 cancers developed in 1,246 individuals who were infected with *H. pylori*.

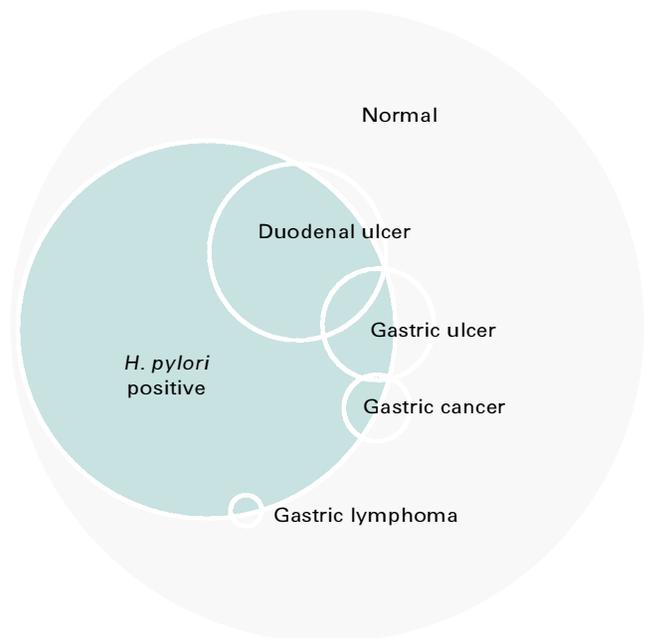
The idea that a whole population could be screened for *H. pylori* and treated on the basis of gastric cancer prevention has been discussed at length by Parsonnet *et al*<sup>27</sup>. Gastric cancer usually occurs after the age of 40, so it is currently not cost-effective to screen and treat children, although there is an effective strategy in the USA in which 50 year old individuals are screened for *H. pylori* and treated if infected.

### Virulence and pathogenicity

*H. pylori* produces large amounts of urease enzyme. By breaking down urea in the gastric juice and extracellular fluid, the organism is able to generate bicarbonate and ammonia in its intracellular and pericellular environment, effectively neutralising hydrogen ions before they can lower the intracellular pH to below 5.0<sup>28</sup>. Thus, when urea is present, *H. pylori* can survive in gastric acid for a time – long enough for it to colonise the gastric mucosa<sup>29</sup>. The external surface of *H. pylori* is coated with an adhesion molecule that attaches to specific receptors on the gastric epithelial cell. The natural home of *H. pylori* is below the gastric mucus, attached to the mucus-secreting epithelial cells that line the stomach. The organism may also be found in the duodenum, where it colonises naturally occurring islands of gastric-secreting epithelium (gastric metaplasia).

Once attached to the gastric epithelial cell, *H. pylori* may cause some damage by producing ammonia, but it also damages the epithelium by causing vacuolation in the epithelial cells. This vacuolation is secondary to the production of a cytotoxin called vacuolating cytotoxin A (VacA), a protein which is endocytosed by epithelial cells where it causes endosome-lysosome fusion (ie vacuoles)<sup>30</sup>. Variants of cytotoxin occur; the more aggressive forms are likely to be associated with peptic ulcer, whereas more benign forms may be associated with gastritis in people who have no symptoms or are without ulcers<sup>31</sup>. Interestingly, this cytotoxin is activated in low pH<sup>32</sup>.

Another important pathogenic factor is the cytotoxin associated gene (*cagA*). This resides within an island of approximately 30 genes, probably acquired by *H. pylori* from another organism since the guanine plus cytosine content of this island differs from that seen in the rest of the *H. pylori* genome<sup>33</sup>. Most of the genes



**Fig 3. Epidemiology and disease associations of *Helicobacter pylori*.** The large circle reflects the whole population within which is a group of about 30% who are infected with *H. pylori* (HP). Most individuals who develop duodenal ulcer, gastric ulcer, gastric cancer and gastric lymphoma are infected with *H. pylori*. These diseases are quite uncommon in uninfected persons. Gastric lymphoma, a low grade lymphoid malignancy of the stomach, usually goes into complete clinical remission when *H. pylori* is eradicated with antibiotics.

in the *cagA* pathogenicity island (*cag* PAI) are part of a structure called a ‘Type IV’ secretion system. This is a derivative of an ancient flagellar structure which is now used to form a tubule through which the CagA protein is transferred to the host epithelial cell<sup>34</sup>. Once inside the cell, tyrosine phosphorylation of the CagA protein allows it to produce a ‘growth factor’ signal; this causes failure of the epithelial cell’s ability to maintain its normal cytoskeletal structure, probably enhancing the attachment and survival of the nearby *H. pylori*<sup>35</sup>.

### Key Points

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**Helicobacters are urease positive microaerophilic mucous adapted**  
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**Helicobacters attach to the gastric mucosa of many species – *H. pylori* is the type strain**  
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**Helicobacters cause gastritis which is an immunological response to infection**  
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***H. pylori* predisposes those infected to peptic ulcer and stomach cancer**  
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***H. pylori* produces toxins directed at epithelial cells**  
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The *cag* PAI also contains genes which induce interleukin (IL)-8 production by the epithelial cells. IL-8 attracts neutrophils which migrate from the capillaries through the lamina propria and emerge between the epithelial cells. The actual cause of duodenal ulcer, the most common type of peptic ulcer, may be the migration of these neutrophils with subsequent release of their digestive products (proteases, reactive oxygen species, etc). *H. pylori* organisms which contain the *cag* PAI usually also have the more active VacA toxin, but it is not known why the two should be associated in this way<sup>36</sup>. There are other less important virulence factors (extensively reviewed by Ge and Taylor<sup>30</sup>).

## Molecular biology and epidemiology

### Genomics

Two complete genomes of *H. pylori* organisms have been published, as well as detailed comparison of the two<sup>14,15</sup>. The function of about 40% of *H. pylori* genes is unknown, but presumably relates in some way to survival in the acid-secreting human stomach. Prior to the genomic study of *H. pylori*, restriction enzyme analyses had shown that the species is extremely diverse – that is, nearly every person on earth has a different restriction pattern for the organism. In fact, this diversity has been exaggerated because most of these changes are ‘synonymous’; they are random mutations in the third base of a codon which do not necessarily alter the resulting protein. Also, *H. pylori* can take up pieces of DNA from its environment as well as shuffle large portions of its genome from time to time. Nevertheless, most of its genes are expressed identically.

Using a whole genome microarray developed from the published genome sequence of *H. pylori*, Salama and colleagues<sup>37</sup> studied 15 unrelated *H. pylori* isolates in order to define the ‘essential genome’ of *H. pylori*. About 22% of the genes in each organism were found to be non-essential whereas 1,281 genes were common to all strains. These studies provide a basis for the development of new therapies for *H. pylori* based on targets essential for its colonisation of the gastric mucosa. Similarly, by directing the immune response towards outer membrane proteins common to all *H. pylori*, preventive, and perhaps eradicated, vaccines might be developed.

### Epidemiology

Molecular epidemiological studies have focused on various types of *vacA* gene and the *cag* PAI<sup>38,39</sup>. Studies of human molecular epidemiology show a probable migration from Asia across Alaska into Latin America more than 10,000 years ago. Initial reports claimed that patterns of *H. pylori* differed from this, and suggested that Latin American *H. pylori* organisms were acquired from Spanish immigrants about 500 years ago<sup>39</sup>. Recent work, however, has revealed that South American tribes in the deep Amazon basin, who have had little contact with European races, sometimes have *H. pylori* related to Asian strains, lending support to the concept of *H. pylori* as an

‘ancient’ organism that travelled with man across the Siberian-Alaskan land bridge some 30,000 years ago<sup>40</sup>.

This has important implications for the controversy about the origins of *H. pylori*. Blaser<sup>41</sup> claims that *H. pylori* is an ‘ancient’ coloniser of man, evolving over millions of years in the human stomach to its present form where most persons are asymptomatic and perhaps live in symbiosis with their *H. pylori* infection. Symbiosis, however, implies that both host and parasite receive some benefit, but the benefits of an asymptomatic *H. pylori* infection seem theoretical and minor at least, whereas the risks of peptic ulcer and gastric cancer still seem significant.

So far, man is the only species known to be commonly infected with *H. pylori*, and the original source of the bacterium is unknown. Recently, tantalising polymerase chain reaction evidence of the organism was found in Sardinian sheep<sup>42</sup>, again stimulating the idea that *H. pylori* is a commensal of some animal species.

### Diagnosis

*H. pylori* can easily be detected by searching for its urease enzyme. When biopsies of gastric mucosa are placed in a gel containing urea, the subsequent ammonia production causes a pH change which is a highly accurate indicator of the infection. An alternative method of detection is for patients to swallow urea labelled with an isotope, either C14 or C13. Detection in the subsequent 10–30 minutes of isotope-labelled CO<sub>2</sub> in the breath indicates that the urea was split – that is, urease and *H. pylori* were present in the stomach<sup>43,44</sup>. As in all bacterial infections, infected persons carry antibodies (usually immunoglobulin G) directed against *H. pylori*. Various laboratory and bedside serological tests exist for detecting antibodies against the organism. These tests have high sensitivity, although they tend to overdiagnose the infection since antibodies remain for some years after adequate treatment<sup>45</sup>. Culture of *H. pylori* usually requires endoscopic biopsy. However, it can also be successfully achieved by patients swallowing a piece of string which can be retrieved after about an hour, revealing a culturable attached organism<sup>46</sup>. Thus, at the present time, patients do not necessarily have to undergo an invasive procedure, and quite inexpensive diagnostic methods are available.

### Treatment

Eradication of *H. pylori* has been shown to be a definitive cure for duodenal ulcer and most gastric ulcers. Gastric cancers usually develop after many years of *H. pylori* infection, and may occur even after the infection has been eliminated naturally or with antibiotic treatment. For this reason, prevention of gastric cancer is an indication for *H. pylori* therapy only in high risk groups (ie Japanese and first-degree relatives of gastric cancer patients).

In the 1980s, treatment for *H. pylori* was difficult since combinations of bismuth, tetracycline and metronidazole (up to 16 tablets per day) were required for adequate eradication of the organism<sup>47</sup>. A breakthrough came when Unge and colleagues in

Sweden<sup>48</sup> observed that the action of amoxicillin was greatly enhanced when gastric acid was suppressed with a proton pump inhibitor, notably omeprazole<sup>48</sup>. Thus, since 1996, it has been possible to treat *H. pylori* relatively easily with a seven-day course of omeprazole (to render the gastric pH neutral) in combination with two antibiotics, usually amoxicillin and clarithromycin. Omeprazole, clarithromycin and metronidazole combinations have achieved similar high cure rates, notably in Italy<sup>49</sup>. For difficult to eradicate infections, bismuth, tetracycline, metronidazole and omeprazole are usually successful<sup>50</sup>. These therapies, and their use in double-blind studies, have proved that peptic ulcer is mostly a bacterial infection unrelated to the victim's emotional state.

Apart from *H. pylori*, the common cause of peptic ulcer is the use of non-steroidal anti-inflammatory drugs (NSAIDs), but their ulcerogenic effect may also be on the wane with the advent of specific cyclooxygenase-2 inhibitor-type NSAIDs which can treat arthritis without necessarily weakening the gastric mucosa<sup>51</sup>.

### Problems for the future

As mentioned above, the exact mode of transmission of *H. pylori* in various societies is not completely understood. In some developing countries, reinfection after treatment is uncommon<sup>52</sup>, whereas in others treatment is reserved only for severe ulcer cases since reinfection is the rule<sup>53</sup>. In areas where *H. pylori* exists in the environment, humans do not seem able to mount a protective immune response following natural infection. Therefore, treatment is useless, and the only effective way of eliminating *H. pylori* from the population would be via public health measures – that is, by improved sanitation and standards of living or vaccination.

### Vaccination against Helicobacter pylori

In the past five years, many groups have been developing potential vaccines that might protect against, or even eradicate, *H. pylori*. Excitement developed when Lee *et al*<sup>54</sup> and Sellman *et al*<sup>55</sup> observed that *H. felis* infection in the mouse (a cat organism infected into mice) could be prevented, and also in some cases eradicated, by vaccination with *H. pylori* antigens in association with small amounts of cholera toxin. Thus, the potential does exist for a similar process in man, whereby individuals are dosed with *H. pylori* antigens via the nasopharynx or upper gastrointestinal tract and caused to develop an immune response superior to that normally seen in *H. pylori* infection. In order to assist development of vaccines, experimental *H. pylori* infections in humans have recently been described<sup>56</sup>, and production of specific antibody in volunteers has been achieved by a Salmonella vaccine strain expressing *H. pylori* urease antigen<sup>57</sup>.

### Conclusions

Twenty years after the isolation of *H. pylori*, with more than half the world still infected, the epidemiology of the infection is a

worthy area of continued study. Once the exact mode of transmission is understood in different communities, effective public health measures can be started. At least in persons with severe manifestations of *H. pylori* infection (ie peptic ulcer, lymphoma and gastric cancer), current knowledge allows effective treatment of the infection. Diagnosis and treatment are still rather expensive for some communities, but the cost is decreasing. It is certainly a considerable cost saving over previous available therapies (H<sub>2</sub>-receptor blockers and surgery) which were the mainstays of treatment for upper gastrointestinal diseases before 1990. In the next decade, it is hoped that advances in immunology and genomics will allow prevention and/or eradication of the *H. pylori* problem with a simple vaccine.

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