# *Helicobacter pylori*: a microbiologist's view

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**Helicobacter pylori *infection is known to cause a number of gastrointestinal diseases. From the standpoint of a microbiologist there are several important facets to this organism, which include the nature of the organism, it's diagnosis and the effectiveness of eradication therapy.***

*Helicobacter pylori* is probably the most widespread bacterial infection of humans and since its description in 1984 (Marshall et al, 1984) it has been the subject of intense interest. The prevalence of *H. pylori* varies widely within and between countries. The highest rates of infection tend to be associated with low socioeconomic status.

Infection is thought to be acquired in childhood, a study showed that the highest seroconversion rates (2.1% per year) occurred in children between the ages of 4 and 5 years. The implication of this finding is that any preventive measures should be aimed at children under the age of 10 years (Malaty et al, 2002).

The genus *Helicobacter* currently contains 31 species, of which 22 are principally associated with extragastric sites (On et al, 2002). *H. pylori*, however, is only found on gastric mucosa and is primarily a human pathogen, although several species of monkeys, nude mice and gnotobiotic piglets have been experimentally infected. Non-humans are also known to have spiral organisms in their stomachs. Ferrets, for example, have *H. mustelae* and cheetahs have *H. acinonyx* but only *H. pylori*, *H. heilmannii* (formerly *Gastrospirillum hominis*) (Solnick et al, 1994) and *H. felis* (Lee and O'Rourke, 1993) have been shown to infect humans. All induce an chronic active gastritis which may or may not be associated with gastrointestinal symptoms.

Failure to consistently isolate *H. pylori* from reservoirs other than man suggests that direct person-to-person contact is the most likely mode of transmission. Strains of the organism show substantial genotypic diversity. A variety of fingerprinting methods have shown that strains which are not epidemiologically linked have different profiles (Akopyanz et al, 1992; Fujimoto et al, 1994). In addition individuals may be simultaneously colonized by more than one strain (Prewett et al, 1992) which suggests that there may be competition between strains for host-derived sites or nutrients (Blaser, 1995).

## BACTERIOLOGY

*H. pylori* is a microaerophilic Gram-negative spiral organism with four to six unipolar flagella (Marshall, 1983). It grows optimally in vitro at 37°C on a variety of basal media with added blood.

One of the most prominent microbiological features of this organism is it's remarkably high urease activity. Bacterial urease hydrolyses urea to ammonia, carbon dioxide and water. The rate of urea hydrolysis in *H. pylori* is more than twice that of *Proteus mirabilis*, another common urease positive bacterium, and around seven times as high as other urease positive coliforms (Mobley et al, 1988). The enzyme has a Km (the concentration of substrate that permits half the maximal rate of reaction) of 0.45 mM (Fujimoto et al, 1994) and this, coupled with the high rate of hydrolysis, is thought to be an adaptation to the relatively low intragastric concentration of urea.

The function of the urease enzyme is thought to be to produce sufficient ammonia to protect the organism from gastric acid. This is supported by in-vitro studies which have shown that the organism can survive at acid pH only in the presence of urea (Marshall et al, 1990). As well as survival benefits for the organism this high urease activity forms the basis of a range of diagnostic tests.

## DIAGNOSTIC TESTS

A variety of methods are available for the diagnosis of *H. pylori* infection. They fall into several groups including urease-based tests, antibody and antigen detection, DNA detection, histology and culture.

### Urease-based tests

These rely on the bacterial breakdown of urea as described. Urea, labelled with either radioactive carbon-14 ($^{14}C$) or the non-radioactive isotope

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$^{13}C$, is given as part of a test meal. The labelled carbon dioxide produced in the stomach is exhaled and detected in the breath by either mass spectroscopy for $^{13}C$ or scintillation counting for $^{14}C$.

Urea breath tests are often considered to be the gold standard for *H. pylori* diagnosis; they may also be used to confirm eradication of the organism (Klein et al, 1996; McNulty and Wyatt, 1999). It is important to remember, however, that the test may yield falsely negative results when used within 2 weeks of proton pump inhibitor or 4 weeks of antibiotic therapy (El-Nujumi et al, 1998). The addition of citric acid to the test meal improves the accuracy of the urea breath test (Rehnberg et al, 2001).

Urease activity may also be detected directly in gastric biopsies. The biopsy is placed in a broth containing urea and a pH indicator. If *H. pylori* is present urease activity leads to a colour change in the pH indicator. This test is both sensitive and easy to interpret but requires an endoscopy to obtain the tissue.

**Antibody tests**

Another group of diagnostic tests aim to detect antibodies to *H. pylori*. The antibody response is predominantly of an immunoglobulin (Ig) G and IgA type (Rathbone et al, 1986) and in a volunteer study IgG seroconversion occurred between 22 and 33 days post infection (Morris and Nicholson, 1987). The detection system used is usually an enzyme-linked immunosorbent assay (ELISA) because of its speed, simplicity and reproducibility, however, the sensitivity and specificity of these ELISA can vary considerably depending upon the 'cut off' value used in the laboratory and the prevalence of *H. pylori* in the population to be tested (Stevens et al, 1997).

Serology may also be used to determine the response to eradication therapy. IgG and IgA titres decline slightly in the first 2 months after therapy but by 6 months after treatment the predictive value of a fall in IgG of 50% is 0.99, and at 12 months the predictive value of a 50% fall is 1.0 (Kosunen et al, 1992). Because of variation in the assays used this comparison is only valid if the original sample is stored and re-assayed at the same time as the follow-up sample, which limits the clinical utility of this method.

In addition to an antibody response *H. pylori* antigen can now be detected in the faeces. Gisbert and Pajares (2002), in a systematic review of 43 studies including 4769 patients, found the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 92.4%, 91.9%, 92.1% and 90.5% respectively. They also reviewed 25 studies including 2078 patients using the test to confirm eradication of *H. pylori*. In this case sensitivity, specificity, PPV and NPV were 88.3%, 92%, 75.1% and 94.8% respectively. Most studies showed that the stool antigen test was an accurate method of confirming *H. pylori* eradication but this was not universal and they concluded that further investigation is necessary before the test can be used with certainty. Again, as with all ELISA type tests, choosing the correct cut off is important as changing this value was shown in one study to increase the sensitivity of the faecal antigen test from 77 to 93% (Leodolter et al, 2001).

**DNA tests**

*H. pylori* DNA can also be detected using the polymerase chain reaction (PCR). This approach has been used with a variety of gene targets in gastric biopsy specimens, however, samples must still be obtained endoscopically.

Amplification technologies are also being applied to faeces and saliva. One study has shown that it is possible to extract *H. pylori* DNA from faeces using a sequence-specific biotinylated oligonucleotide capture probe with subsequent magnetic separation of target DNA complex sequences (Shuber et al, 2002). A modification of this technique has also been applied to the stools of children and has shown a PPV of 80% in this epidemiologically important group (McKay et al, 2003). The advantage of molecular methods is that they will enable genotyping studies to be undertaken to clarify the mode of transmission of the organism.

PCR analysis of dental plaque from subjects with gastric infection has yielded conflicting data, with *H. pylori* DNA found frequently in some studies (Banatvala et al, 1994) but absent or found at extremely low frequencies in other studies (Hardo et al, 1995). As such the hypothesis that the oral cavity may be a permanent reservoir of viable *H. pylori* still remains a controversial issue.

**Bacterial culture and histology**

The mainstay of diagnostic testing used to be bacterial culture and histology. In clinical practice the bacterium is now rarely cultured from endoscopic biopsies. If culture is required *H. pylori* grows on a wide range of basal media supplemented with 5–10% v/v blood in an atmosphere of 5% oxygen with 5–10% carbon dioxide.

One problem with the change to non-culture-based methods of diagnosis is that data on the development of antibiotic resistance are less widely available. The prevalence of resistance varies both with antibiotic and geographical location. A study showed the overall metronida-

zole resistance rate to be 40% (Parsons et al, 2001). Resistance is also more common in women and the young, but does not appear to be related to socioeconomic status.

Clarithromycin resistance in the general population in France and Belgium, where macrolide consumption is higher, is around 15% (Dupas, 2003) and may be as high as 69% in patients following treatment failure (Tankovic et al, 2001). No large studies are available for the UK but one study performed here showed that 4 of 90 *H. pylori* isolates were clarithromycin resistant (Warburton-Timms and McNulty, 2001)

Clarithromycin resistance has been shown to significantly reduce the effectiveness of clarithromycin-containing eradication regimens (Poon et al, 2002; Miki et al, 2003). This effect is less marked with metronidazole resistance but this may be a result of methodological problems in determining resistance to metronidazole (Moayyedi et al, 1998). Worryingly evidence is beginning to appear that resistance rates for both clarithromycin and metronidazole reflect the annual consumption of these agents in the population (Perez Aldana et al, 2002). As such any increased use of macrolide antibiotics overall may limit the effectiveness of macrolide-based treatments used as part of a 'test and treat' strategy.

## VIRULENCE FACTORS

Most individuals with *H. pylori* infection are asymptomatic. This has prompted a search for bacterial virulence factors which could be used to predict the development of peptic ulcer disease or gastric cancer.

Adherence to gastric epithelial cells is a prerequisite for both colonization and pathogenesis. In this context there has been considerable interest in *H. pylori* strains which express blood group antigen as increased bacterial loads have been found in patients infected with strains expressing the blood group A antigen-binding adhesin (BabA). The higher bacterial load was also associated with an increased interleukin 8 (IL-8) response and higher granulocytic infiltration, which may lead to enhanced mucosal damage (Rad et al, 2002).

When Lewis antigens are studied along with other virulence factors such as as CagA and VacA it is possible to define a cluster of bacterial parameters which are significantly associated with the development of atrophic gastritis (Broutet et al, 2002). This is of clinical relevance as there may be an increased risk of gastric cancer in *H. pylori*-infected patients with atrophic gastritis (Uemura et al, 2001).

Of the other virulence factors, one of the first to be identified was the CagA protein. CagA proteins are a group of antigenically related surface associated proteins with a molecular weight of 120–140 kDa. The gene for these cytotoxin-associated proteins has been designated cagA (Tummuru et al, 1993). It is now known that the CagA protein is a marker for the presence of a pathogenicity island of virulence determinants, the presence of which has been associated with more severe disease in a variety of studies. In many Asian countries, however, the prevalence of the cag pathogenicity island is nearly 100% regardless of the clinical disease manifested by the patient.

iceA (induced by contact with the epithelium) is another virulence factor which exists in at least two allelic forms, iceA1 and iceA2. Adherence to gastric epithelial cells in vitro stimulates the transcription of iceA1 and strains positive for iceA1 have been associated with both peptic ulceration and increased mucosal concentrations of IL-8 (Sheu et al, 2002).

The third major virulence factor identified is vacuolating cytotoxin. Supernatants from cultures of some strains of *H. pylori* induce vacuole formation in eukaryotic cells (Leunk, 1991).

This cytotoxin has been identified as a 87 kDa extracellular protein (Cover and Blaser, 1992). The gene encoding this protein has been identified and is known as vacA (Telford et al, 1994). This gene is present in nearly all strains of *H. pylori* examined but only a proportion express the cytotoxin. Most of the gene is conserved, however, the middle of the gene shows some differences between those strains expressing cytotoxic activity (tox+) and those which do not (tox-) (Cover et al, 1994).

An association between the expression of CagA protein and cytotoxic activity has also been observed (Hardo et al, 1995). Almost all tox+ strains are CagA+ and are known as type I; the vacA- and CagA- strains are known as type II. However, there is no complete correlation between any of the virulence factors and disease, indeed the role of all of the putative virulence factors in the causation of disease remains uncertain.

## CONCLUSION

Knowledge about *H. pylori* infection has expanded greatly over recent years. A range of diagnostic tests are now available and indications and regimens for treatment are more defined. However, many aspects remain unclear including the relationship between the organism and its local environment and mechanisms of pathogenicity. There is much more to be discovered about the nature of this ubiquitous organism. HM

*Conflict of interest: none.*

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## KEY POINTS

- *Helicobacter pylori* remains an important pathogen which is becoming more resistant to antibiotic therapies.
- Infection is thought to be acquired in childhood and transmission is thought to be by the faecal oral route.
- There are an increasing number of tests available for the diagnosis of this organsism, they include urea breath testing, antibody detection in blood and antigen detection in faeces.
- A number of putative virulence factors have been described but as yet an 'ulcerogenic strain' of the organism has not been identified.

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