# Helicobacter pylori: Past… Present… Future

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Helicobacter pylori is a small, spiral-shaped, highly motile gram negative bacterium that is related to Campylobacter and colonises non-acid secreting musoca of the stomach and upper intestinal tract [1]. It is urease, catalase, and oxidase positive. Originally called Campylobacter pyloridis and then corrected to Campylobacter pylori, the bacteria were renamed again due to taxonomic data as Helicobacter pylori in a new genus, Helicobacter.

Infection with H.pylori is very common, with approximately 50% of the world's population infected [2]. Once present, infection will often become chronic and persistent and evidence shows strong correlation between its presence and gastrointestinal diseases like gastritis, peptic ulcer disease, gastric carcinoma and MALT lymphoma [3].

## History

H. pylori was discovered by Marshall and Warren in 1982 {4] resulting in what was at the time, a divergence from the archetypal understanding of gastric disease. It was commonly thought that stress and diet were the only causes of peptic ulcers however the work of Marshall and Warren identified and isolated Campylobacter-like organisms (CLO) in ulcer biopsies. This discovery was met with much scepticism and resulted in an infamous example of tenacity and scientific endeavour. In 1985 Marshall performed self-inoculation by CLOs and exhibited symptomatic gastritis, which he subsequently treated successfully with metronidazole and bismuth salts, thereby proving their ability to cause gastritis [5]. Their work on H. pylori and the resulting paradigm-shift in the understanding of gastric disease led to them being awarded the Nobel Prize for Medicine in 2005.

## PATHOLOGY

H. pylori is considered a type I carcinogen and is the most common cause of infection-related cancers, representing 5.5% of the global cancer burden [6].

While in most cases infection with the bacteria is asymptomatic, long-term carriage significantly increases the risk of developing diseases. Studies have reported approximately 10% develop peptic ulcer disease, 1 to 3% develop gastric adenocarcinoma, and <0.1% develop mucosa-associated lymphoid tissue (MALT) lymphoma [7]. The pathogenicity of H. pylori and subsequent risk of cancer is dependent on both the bacterial and host genotypes as well as environmental exposures [8].

Two loci play a part in determining the virulence of H. pylori; the cag pathogenicity island (cag PAI) and vacA. The cag PAI encodes the CagA protein, often used to broadly differentiate between strains, which is tyrosine phosphorylated inside the host cell resulting in increased cellular migration and has been linked to oncogenesis [9]. As well as encoding for CagA, cag PAI also delivers H. pylori peptidoglycan to the host cells triggering an intracellular signaling cascade, which culminates in the production of type I interferon (IFN), an important group of proteins involved in regulation of the immune system [10]. The toxin VacA encoded by the vacA locus, also has a role to play in modulation of the immune system and inflammatory response.

While the relative virulence of H. pylori can be identified by analysis of the bacterial genotype, there are a number of host factors that affect the development of H. pylori-induced carcinogenesis including gastric inflammation and a reduction in acid secretion [11].

Interestingly, it has also been postulated there is a synergistic relationship between high salt diets and H. pylori infection on gastric inflammation and damage. The link has been studied in gerbils [12] however the mechanisms of action are not fully understood. Some hypotheses point to a link between salt and its effect on gastric mucosa and epithelium allowing carcinogens to pass into gastric tissue and facilitating malignant transformation, while other studies link salt to increased inflammation and upregulation in cytokines such as interferon. One more recent study observed a potential correlation between high gastric salt concentration and modulation of gene expression in H. pylori [13].

## Diagnosis

A number of techniques have been developed to diagnose H.pylori infection and can be grouped broadly into invasive and non-invasive methods.

Invasive methods include culture and histology, and require accessing the stomach either by endoscopy or an alternative such as nasogastric tube or oro-gastric brush and obtaining a biopsy. The endoscopic features of H. pylori infection are not specific and difficult to detect using standard methods, however improvements in imaging and microscopy have led to better detection and subsequently better biopsy samples being obtained [14]. Several tests can be performed on the gastric mucosa biopsy: Rapid urease test (RUT), histology, smear (cytology), culture and polymerase chain reaction.

The RUT is similar in principle to the urea breath test described below, however it requires a sample of gastric mucosa or mucus which is brought into contact with urea and the hydrolysis artefacts are detected. The initial test used phenol red which changes from yellow to pink or red as the pH increases due to $CO_2$ production [15]. This method was evaluated in detail in 1989 by McNulty et al and found to be a cheap and rapid alternative to staining or culture of biopsy samples [16].

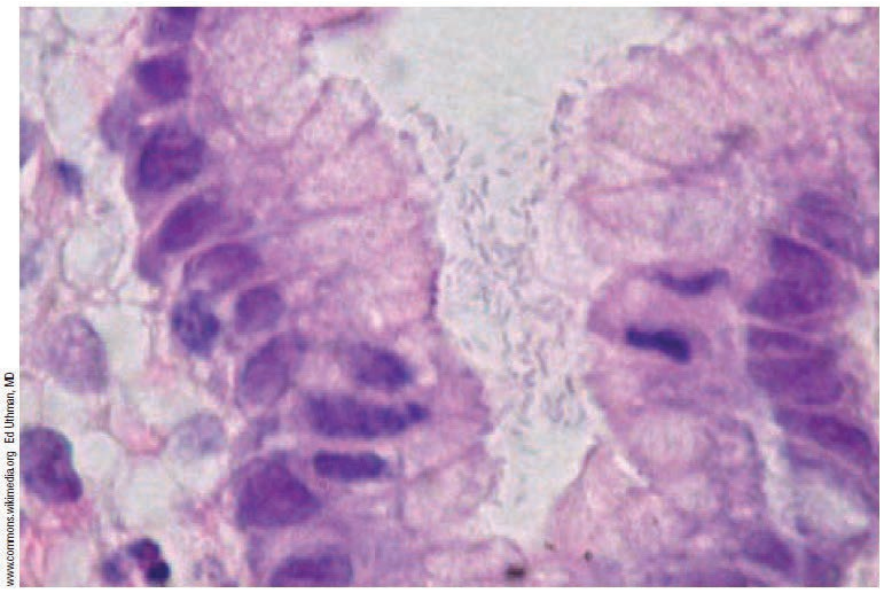


*Figure 1. Numerous curved Helicobacter pylori fill the lumen of a gastric gland (haematoxylin and eosin stain, original magnification x1000).*

A number of staining methods are available for histological investigation of biopsy samples for H. pylori, most commonly a routine haematoxylin and eosin (HE) stain. In the UK according to the latest guidelines from Public Health England, microscopy is carried out using carbol fuchsin or Sandiford's stain. Staining and examination of the stained

preparation using Gram or Giemsa stains need only be performed if the culture result is negative and the biopsy urease test positive [17].

Cytology, and more specifically, imprint cytology has been evaluated as a cheap, rapid alternative to traditional histological investigation. Biopsy specimens are rolled out onto a clean glass slide to form an imprint smear and air dried. Staining is then performed and this method has been found to have a sensitivity and specificity equal to that of histology [18].

H. pylori culture can be performed on selective agars which contain specific antibiotics to inhibit commensal bacteria, and nonselective agars. Culture must be performed as soon as possible after sampling and incubated under microaerobic conditions (85% N2, 10% CO2, 5% O2) at 35 to 37°C for at least 7 days before discarding cultures as negative [19].

The application of PCR on gastric biopsy samples for the identification of H. pylori was first described by Hammar et al in 1992 [20]. The assay targeted a DNA region coding for a species-specific protein antigen which was present in all strains of H. pylori. Since then methods for extracting the bacterial DNA from faecal samples, which consist of a complex matrix often including PCR inhibitors, as well as the overall decreasing cost, availability and ease of use of molecular methods has improved PCR testing, however as with all molecular tests there is a risk past infections will be identified leading to false positive results [21]. This being said, an advantage of PCR is the ability to identify genes relating to antimicrobial resistance mechanisms which will be covered later in this review.

Non-invasive methods include serological testing, antigen testing from faecal samples and the urea breath test.

First described in 1987, the urea breath test detects labelled carbon dioxide in expired air as a result of urease production by H.pylori [22]. Urease hydrolyses urea into ammonia and $CO_2$. Patients are administered urea labelled with an uncommon carbon isotope, either radioactive carbon-14 or non-radioactive carbon-13. Once the urea is hydrolysed the carbon isotope in CO2 can be measured and detected during exhalation.

Blood serology is understood to be the least accurate method as it detects antibody to helicobacter and does not differentiate active from past infection, however a review conducted by Leheji et al showed kits detecting IgA, IgG, and IgM simultaneously or IgA alone do not perform as well as those which detect only IgG antibodies [23].

Data comparing the performance of the urea breath test and the stool antigen test is summarised in the table below:

*Table 1. Data from Luciana Cardinali et al. J Clin Microbiol. 2003 July; 41(7): 3334–3335.*

| | Urea Breath Test | Stool Antigen Test |
|---|---|---|
| Sensitivity (%) | 93.8 | 96.9 |
| Specificity (%) | 99.1 | 100 |
| PPV (%) | 97.8 | 100 |
| NPV (%) | 98.0 | 98.0 |

Antigen testing can be performed both by enzyme-linked immunosorbent assay (ELISA) or rapid lateral flow methods. The stool antigen test allows collection of the sample at home and is usually recommended when the UBT is not available [24]. Both methods use antibodies against H. pylori antigen to detect presence of the bacteria, however performance of commercial kits has been shown to be uneven [25].

The cost of each of these tests is a factor in the choice of diagnostic method used in the laboratory. The urea breath test is the most expensive, followed by stool antigen and finally blood serology. A limitation of urea breath test and stool antigen test is the need to cease treatment of proton pump inhibiters (within two weeks of testing) and antibiotics (within four weeks of testing) as these drugs suppress bacteria and may lead to false negative results [26].

## Treatment

In the UK, NICE guidelines for first-line treatment of H. pylori are a 7 day, twice-daily course of treatment with: a Proton Pump Inhibitor plus two antibiotics; amoxicillin and either clarithromycin or metronidazole. Patients still experiencing symptoms after 7 days are offered a second-line treatment with: a Proton Pump Inhibitor plus amoxicillin and either clarithromycin or metronidazole (whichever was not used first-line). This triple therapy has been the standard treatment for H. pylori infection for the past 15 years however there has been an increase in antimicrobial resistance which is causing concern over the efficacy of this treatment.
A recent review of treatment options for clarithromycin resistant H. pylori has indicated a return to the initial treatment by Marshall and Warren using bismuth. In the form of bismuth subcitrate, it's mechanisms of actions are not fully understood but it has been shown to have anti-inflammatory and bactericidal action [27]. Initially testing for clarithromycin resistance has been recommended to determine if the traditional triple treatment will be effective; if the strain causing infection is found to be resistant it is recommended metronidazole is administered however resistance rates to metronidazole have also been increasing. A regimen containing levofloxacin is an effective alternative, and as such, it should be used wisely to avoid development of drug resistance [28]. It has also been suggested that a bismuth-based quadruple therapy, PPI plus a standardised three-in-one capsule, bismuth subcitrate potassium, metronidazole, and tetracycline (BMT, sold under licence as Pylera®) is used [29].

As discussed previously, PCR is a useful tool for identification of H. pylori and this is becoming all the more apparent with the increase in drug resistant strains. A method using nested-PCR targeting the 23S rRNA gene was described by Rimbara et al in 2013 [30]. This method offers detection of clarithromycin resistance gene from faecal samples, gastric juice or biopsy material which, as indicated above, is a clinically useful tool for determining patient treatment. Traditional antibiotic susceptibility testing methods using disc diffusion or broth microdilution may also be used to identify susceptibility/resistance to various antibiotics.

## Conclusions

Invasive testing methods are unfeasible for routine testing in laboratories, and while of use in specific cases, non-invasive alternatives should be used. It is clear from looking at existing data that blood serology testing, while being a cheaper option, does not lead to long term savings with regard to patient care and treatment options due to its poor performance characteristics. The urea breath test is expensive and does not offer much in the way of benefits with regard to sensitivity and specificity which in turn impacts the long term cost savings in patient care. Stool antigen testing seems to combine performance and cost effectiveness. However, with the increase of antimicrobial resistance it is becoming more important to conduct further testing once H. pylori infection has been confirmed to provide clinicians with the resistance/susceptibility profiles required to make effective treatment decisions.

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