Helicobacter pylori: past, present and future

Although the word ubiquitous is often over-used, its use as a descriptor in relation to *H. pylori* infection is apposite. Here, Charlotte Duncan provides an overview of a bacterium responsible for a wide spectrum of upper gastrointestinal disease.

Helicobacter pylori is a small, spiral-shaped, highly motile Gram-negative bacterium related to *Campylobacter* which colonises the non-acid-secreting mucosa 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*, it was 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 persist, with evidence showing strong correlation between its presence and gastrointestinal diseases such as gastritis, peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue (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 ulcer; 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 in Physiology or Medicine in 2005.

PATHOLOGY

H. pylori is considered a type I carcinogen and is the most common cause of infection-related cancer, representing 5.5% of global cancer burden.[6] While most cases of infection are asymptomatic, long-term carriage significantly increases the risk of developing disease. Studies have reported approximately 10% develop peptic ulcer disease, 1–3% develop gastric adenocarcinoma, and <0.1% develop MALT lymphoma.[7] The pathogenicity of *H. pylori* and subsequent risk of cancer is dependent on 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 differentiate between strains, which is tyrosine phosphorylated inside the host cell, resulting in increased cellular migration and a link to oncogenesis.[9] As well as encoding for CagA, *cag* PAI also delivers *H. pylori* peptidoglycan to host cells, triggering an intracellular signalling cascade that 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.

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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 been postulated that 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 thus facilitate malignant transformation. Other studies link salt to increased inflammation and up-regulation of cytokines such as interferon. A 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 histopathology, and require accessing the stomach lining for biopsy, either by endoscopy or an alternative such as nasogastric tube or orogastric brush. The endoscopic features of *H. pylori* infection are non-specific and difficult to detect using standard methods; however, improvements in imaging and microscopy have led to better detection and subsequently better biopsy samples.[14] Several tests can be performed on a gastric

mucosa biopsy: rapid urease test (RUT), histopathology, smear (cytopathology), culture and polymerase chain reaction (PCR) methods.

The RUT is similar in principle to the urea breath test (UBT) described below; however, it requires a sample of gastric mucosa or mucus, which is brought into contact with urea, resulting in the detection of hydrolysis products. 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*., who found it to be a cheap and rapid alternative to the staining or culture of biopsy samples.[16]

A number of staining methods are available for the histological investigation of biopsy samples for *H. pylori*, with the most common being a routine haematoxylin and eosin (HE) stain. In the UK microscopy is carried out using carbol fuchsin or Sandiford's stain. Staining and examination of the preparation using Gram or Giemsa stains need only be performed if the culture result is negative and the biopsy urease test positive.[17]

Cytopathology, more specifically imprint cytopathology, has been evaluated as a cheap, rapid alternative to traditional histological investigation. Biopsy specimens are 'rolled' on a clean glass slide to form an imprint smear, then air dried and stained. This method has been found to have a sensitivity and specificity equal to that of histopathology.[18]

H. pylori culture can be performed on selective agars, which contain specific antibiotics to inhibit commensal bacteria, and on non-selective agars. Culture must be performed as soon as possible after sampling and incubated under microaerobic conditions (85% N_2, 10% CO_2, 5% O_2) at 35–37˚C for at least seven days before discarding as negative.[19]

The application of PCR methods to 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 present in all strains of *H. pylori*. Subsequently, methods for extracting bacterial DNA from faecal samples, which consist of a complex matrix often including PCR inhibitors, at overall decreasing cost, availability and ease of use of molecular methods, has improved PCR testing. As with all molecular tests, however, there is a risk that past infection will be identified, leading to false-positive results.[21] On the other hand, an advantage of PCR is the ability to identify genes relating to antimicrobial resistance mechanisms.

Non-invasive methods include serological testing, antigen testing from faecal samples and the UBT. First described in 1987, the UBT 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 [^{14}C]-urea or non-radioactive [^{13}C]-urea. Once the urea is hydrolysed the carbon isotope in CO_2 can be measured and detected during exhalation.

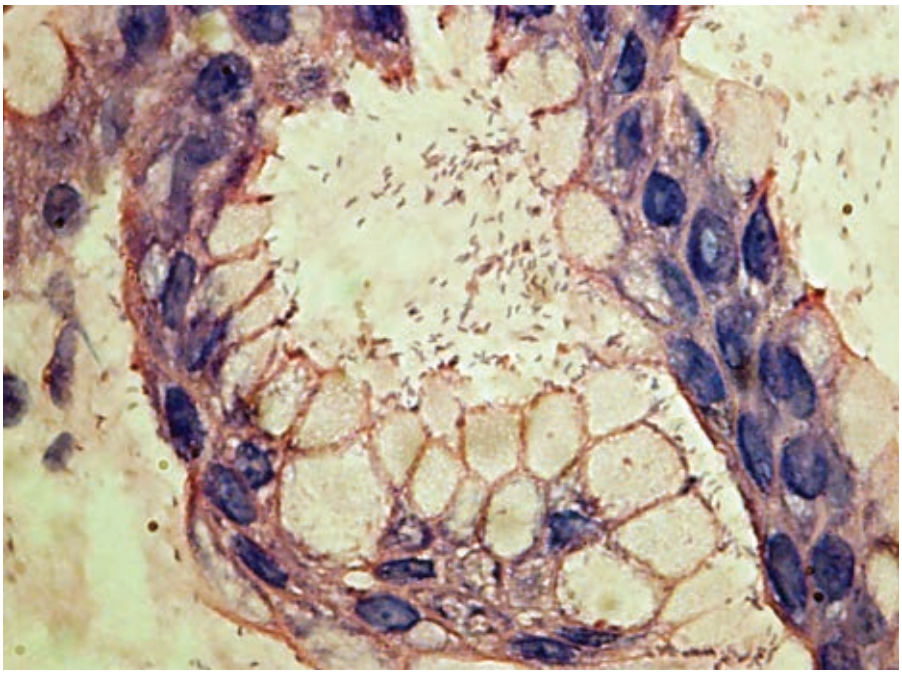

Fig 1. *H. pylori* in the gastric mucosa (progressive Giemsa method; 1 in 15 dilution, pH 2.94).

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 that detect only IgG antibodies.[23]

Data comparing the performance of the UBT and the stool antigen test are summarised in Table 1.

Antigen testing can be performed 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 UBT is not available.[25] Both methods use antibodies against *H. pylori* antigen to detect the presence of the bacteria, but performance of commercial kits has been shown to be uneven.[26]

The cost of these tests is a factor in the choice of diagnostic method used in the laboratory. The UBT is the most expensive, followed by stool antigen and finally blood serology. A limitation of UBT and stool antigen test is the need to cease treatment with proton pump inhibiters (PPI; within two weeks of testing) and antibiotics (within four weeks of testing), as these drugs suppress bacteria and may lead to false-negative results.[27]

Table 1. Performance of the urea breath test and stool antigen test.[24]

	Urea breath test	Stool antigen test
Sensitivity (%)	93.8	96.9
Specificity (%)	99.1	100
PPV (%)	97.8	100
NPV (%)	98.0	98.0

TREATMENT

In the UK, the National Institute for Health and Care Excellence (NICE) guidelines for first-line treatment of *H. pylori* include a seven-day, twice-daily course of treatment with a PPI plus two antibiotics; amoxicillin and either clarithromycin or metronidazole. Patients still experiencing symptoms after seven days are offered a second-line treatment with a PPI plus amoxicillin and either clarithromycin or metronidazole. This triple therapy has been the standard treatment for *H. pylori* infection for the past 15 years. There has, however, 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* by Marshall and Warren suggests a return to the initial treatment using bismuth in the form of bismuth subcitrate. Its mechanisms of actions are not fully understood but it has been shown to have anti-inflammatory and bactericidal action.[28] Initially, testing for clarithromycin resistance has been recommended to determine if traditional triple treatment will be effective; if the strain causing infection is found to be resistant then metronidazole administration is recommended; however, resistance rates to metronidazole have also been increasing.

A regimen containing levofloxacin is an effective alternative, but should be used wisely to avoid development of drug resistance.[29] It has also been suggested that a bismuth-based quadruple therapy comprising a PPI plus a standardised three-in-one capsule containing

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bismuth subcitrate potassium, metronidazole, and tetracycline (BMT; available under licence as Pylera) be used.[30]

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 ribosomal RNA (rRNA) gene was described by Rimbara et al. in 2013.[31] This method offers detection of the clarithromycin-resistance gene from faecal samples, gastric juice or biopsy material, which 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 not feasible options for routine testing in laboratories, and therefore non-invasive alternatives should be used. It is clear from 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 UBT is expensive and does not offer much in the way of benefits with regard to sensitivity and specificity, which impacts on the long-term cost savings in patient care. Stool antigen testing seems to combine performance and cost-effectiveness. However, with increase in antimicrobial resistance, it is becoming more important to conduct further testing once *H. pylori* infection has been confirmed, providing clinicians with the resistance/susceptibility profiles required to make effective treatment decisions. ■

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Charlotte Duncan is a product specialist at Pro-Lab Diagnostics.