Human physiology (II) laboratory

Lab 8: Helicobacter pylori detection

1-Introduction

Helicobacter pylori is a Gram-negative, microaerophilic spiral bacterium which is recognized as the **primary cause of chronic gastritis in humans**. Untreated H. pylori infection precedes to the development of most cases of gastric and duodenal ulcers. Further, H. pylori infection substantially increases the risk of development of gastric cancer in some populations. It is now recognized as probably the most common bacterial infection of humankind, infecting approximately 50% of the world's population. Specific antibiotics combined with proton pump inhibitors are now routinely used to treat and eliminate previously chronic gastroduodenal diseases.

Chronic infection with H. pylori is now recognized as a significant risk factor for gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The prevalence of H. pylori infection, gastritis, and gastric carcinoma increases with age.

The mode of transmission of H. pylori is unclear, although both the fecal-oral and oral-oral routes are likely. There is evidence suggesting that transmission occurs between members of the same family. A definitive natural reservoir of H. pylori external to the human body has not been identified.

2-H Pylori Virulence Factors

A major virulence factor of H pylori is the production of the protein **CagA**, which is **highly immunogenic.** The organism has the ability to inject the CagA protein into the gastric epithelial cells. Once the CagA protein is in the epithelial cells, changes occur in the function of the cell's signal transduction pathways and in the structure of the cytoskeleton.

A second virulence factor is vacuolating cytotoxin, or VacA. The VacA gene **codes for a toxin precursor.** Epidemiological studies have shown that if the CagA and VacA genes are present in the strain of bacteria infecting the individual, there is a higher risk of developing gastric or peptic ulcers or gastric carcinoma.

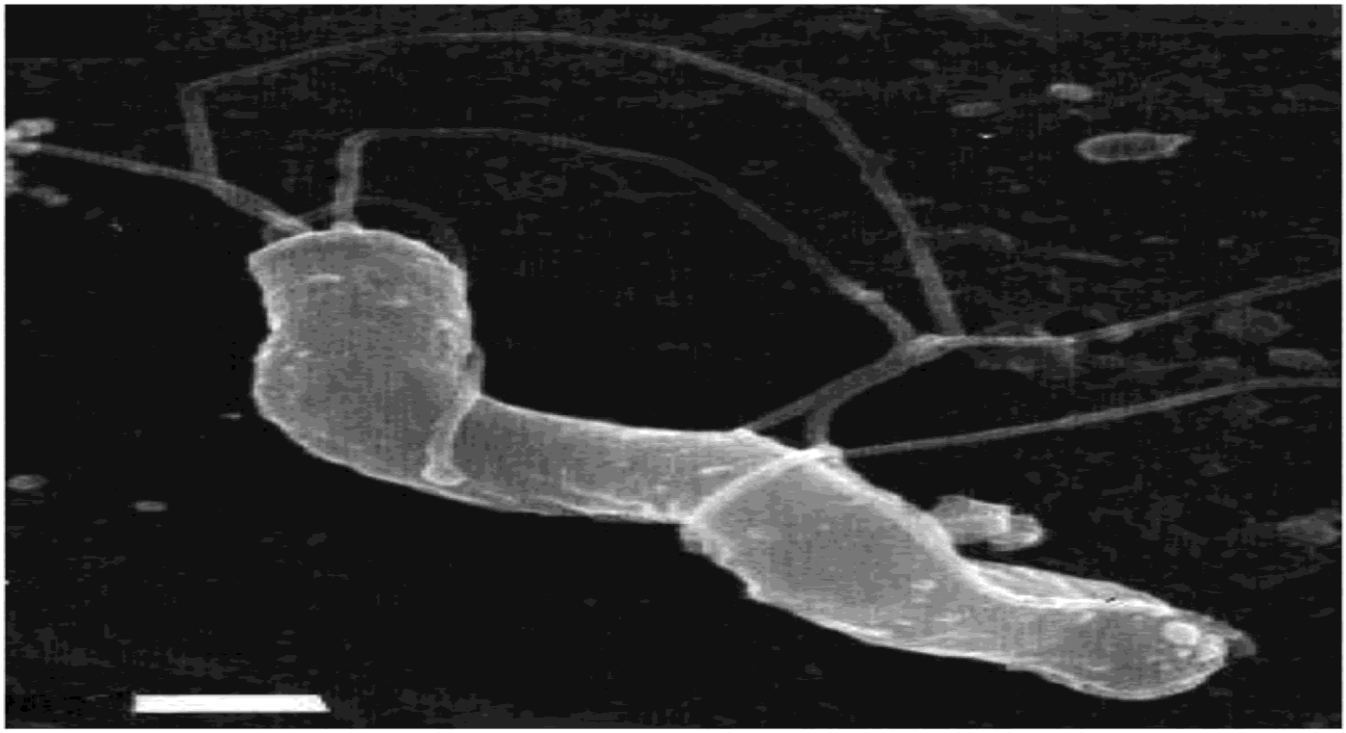
More recently, researchers have focused on a variety of additional virulence factors, including the iceA, babA, oipA, and sabA genes. In addition, an array of pro-and anti-inflammatory cytokine polymorphisms in the host are known to affect the infectious outcome.

3-Pathology and Pathogenesis

Unlike many other bacteria, H pylori can survive and multiply in the gastric environment. This occurs because of several characteristics of the bacteria. Its spiral shape and flagella help the organism to be highly mobile and to penetrate the viscous mucus layer in the stomach. The organism produces large amounts of urease, providing a buffering zone around the bacteria that protects it from the effects of the stomach acid. In addition, the acid-labile flagella are coated with a flagellar sheath, protecting them from the acidic environment of the stomach.

The pathology and mechanism of action leading to tissue damage is not clearly understood. Neutrophil-induced mucosal damage may be the result of the ammonia produced by urease.

Not all individuals harboring the organism go on to develop disease, suggesting that the interaction between the host (perhaps because of genetic predisposition) and the bacteria play a role



4-METHODS FOR DETECTION OF H. PYLORI

Detection of H. pylori infection can be achieved through invasive methods such as endoscopy and biopsy or noninvasive techniques like serological analysis, fecal antigen detection, and urea breath tests. Culture is the most specific test, but it has lower sensitivity due to uneven bacterial distribution in gastric tissue. Diagnostic methods are categorized into:

1-Endoscopy and biopsy are expensive and invasive but provide valuable insights into gastric lesions. A common diagnostic method involves detecting urease from a stomach antrum biopsy. The CLOtest, a widely used test, detects urease activity in gastric mucosal biopsies. During endoscopy, a small tissue sample (1–3 mm) is taken and placed in a test cassette. If urease is present, the yellow gel turns hot pink due to pH changes, indicating a positive result. Most tests yield results within 20 minutes, but low-level infections may require 24 hours for detection. The CLOtest is easy to use and ideal for rapid H. pylori diagnosis.

Noninvasive Detection Methods for H. pylori

Techniques that avoid endoscopy include:

1-Urea Breath Test (UBT)

Procedure: Patient ingests urea labeled with radioactive (^{14}C) or nonradioactive (^{13}C) carbon.

Mechanism: H. pylori metabolize urea to ammonia and bicarbonate. Bicarbonate is exhaled as labeled carbon dioxide and detected by radioactivity (^{14}C) or mass spectrometry (^{13}C).

Applications:

High sensitivity and specificity.

Useful for confirming eradication post-treatment.

Concerns regarding radioactive use in some cases.

2-Stool Antigen Testing

Mechanism: Detects H. pylori antigens in feces.

Sample Collection:

A fresh stool sample is collected from the patient in a sterile container.

Proper storage conditions (refrigeration or freezing) should be maintained if there is a delay in testing.

Procedure

Sample Preparation:

The stool sample is processed to extract antigens, often by mixing a small portion of the sample with a buffer solution to create a testable suspension. Uses:

Evaluates treatment success by analyzing pre- and post-treatment samples.

Not recommended for initial diagnosis due to possible asymptomatic carriage.

Limitations:

False negatives may occur if the stool sample is improperly stored or collected during antibiotic or proton pump inhibitor (PPI) therapy.

Requires proper timing post-treatment (e.g., 4 weeks after antibiotics) to ensure accuracy.

3-Molecular Testing (PCR)

Detects H. pylori DNA in fecal samples.

Limitations:

Cannot differentiate between live and dead bacteria And approved assays not yet available.

4-Serological Testing

Detects antibodies (primarily IgG) against H. pylori.

Key Points:

IgG is the most reliable indicator.

IgA sensitivity is lower; combining IgG and IgA increases detection accuracy.

IgM is not clinically valuable due to chronic nature of infections.

A decline in antibody titers post-treatment indicates successful therapy.

Methods: ELISA, immunoblot, and lateral flow assays (LFA).

ELISA is the preferred method due to high sensitivity and specificity.

Tests using antigens from diverse H. pylori strains are most effective.

Limitations:

Antibody testing unsuitable for confirming eradication due to persistence of antibodies post-treatment.

False negatives possible in immunocompromised individuals.

Applications: Cost-effective for initial diagnosis in untreated patients.

Test(s)	Advantage(s)	Disadvantage(s)	Approximate sensitivity, specificity (%)
Histology	Allows evaluation of the type and degree of inflammation. required to determine whether malignancy is present	Results may vary, depending on pathologist experience. multiple biopsies are recommended	>75, >95
Culture	Allows strain characterization, including identification of antibiotic-resistant isolates and virulence factors	Results vary depending on gastric sampling and laboratory experience; considerable risk of false-negative results	>75, 100
Biopsy urease test	Rapid and inexpensive; endoscopic method of choice	May be falsely negative immediately after antibiotic treatment or during treatment with omeprazole	>90, >95
Rapid urease test	Results available within 1 h	As above, sensitivity and specificity are reduced compared to those of a biopsy urease test	~90, ~90
Urea breath tests using 14C-or 13C-labeled urea	Noninvasive; sealed breath samples can be sent to reference laboratories; the [13C] urea breath test is preferred for children and when multiple tests are required; useful to assess eradication of H. pylori	[14C] urea breath test involves low-level radiation exposure: [13C] urea breath test requires specialized equipment (mass spectrometer)	>95, >95
Stool antigen	Noninvasive; monoclonal antibody-based tests give best results; useful to assess eradication of H. pylori	May be less sensitive in detecting eradication than urea breath test.	>90, >95
Serology	Noninvasive; inexpensive and available commercially. suitable for population prevalence studies	Prolonged delays before antibody levels decrease after eradication	>95, >90
Urine antibody	Noninvasive; easy to obtain especially in children	Not available universally	>85, >90
Salivary antibody	Noninvasive; results are easy to obtain, especially for children	Not available universally	>80, >80
Molecular tests	Allow sensitive detection of H. pylori and specific virulence and antibiotic resistance markers	Not well standardized or available in routine clinical laboratories	Variable