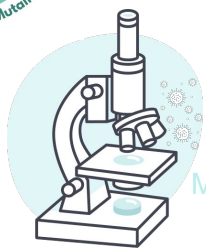


TEAM443
MICROBIOLOGY

H pylori and drugs used in treatment

Dr. Khalifah & Prof. Fawzia

Objectives

- Explain the various gastric and duodenal diseases caused by H.pylori.
- Discuss the epidemiology, transmission of H. pylori and preventative methods used for H. pylori infection..
- Describe the pathophysiology of H.pylori inside the stomach and duodenum.
- Define peptic ulcer disease and assess its distribution among patients.
- Indicate the signs and symptoms of associated disease.
- Discuss the impact of the discovery of H.pylori on the change of diagnosis and management of peptic ulcer
- Describe laboratory characteristics of H.pylori, its identification and diagnosis
- Describe the management and treatment regiments used for eradication of H.pylori

Any future corrections will be in the editing file, so please check it frequently

Osmosis video

Color Index:
Main text
Important
Doctor Notes
Males slide
Females slide
Extra

Overview about Helicobacter Pylori

Helpful video

Discovered in 1983 in Perth (Australia), by Warren and Marshall. Their discovery revolutionised the treatment of duodenal and gastric ulcers. It earned them the Nobel prize for Medicine in 2005

Nearly 20 species of Helicobacter are now recognised.

H. pylori are found in the human stomach. {1}

There is no evidence of animal-to-human transmission. {2}

Epidemiology of Helicobacter Pylori

Around 50% of world's population harbor H pylori.

Over 80% of individuals infected with the bacterium are **asymptomatic.** {3}

Third world has more rate of infection & Infection is more prevalent in developing countries. {4}

Infections are usually acquired at childhood.

Poor sanitary conditions contribute to high rates.

Overall frequency of H pylori infection is declining.

In USA high prevalence among African-American and Hispanic population, due to **socioeconomic status.**

Prevalence varies greatly among countries and population groups

The route of transmission is unknown, although it is known individuals typically become infected in childhood.

Higher hygiene standards and widespread use of antibiotics behind lower rate of infection in the west.

Disease caused by H Pylori

Helicobacter pylori is found closely associated with gastric mucosa and is an independent risk factor for the development of: {5}

- Chronic or acute active gastritis
- Gastric & Duodenal ulcer (Peptic ulcer) {6}
- Gastric adenocarcinoma {7}
- Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

Transmission {8}

Contagious with an **unknown** route of transmission.

Person to person (oral to oral or **fecal-oral)** route.

Transmission occur mainly within families or community.

Gastric antrum of the stomach is the most favoured site of colonization.

Increases oral-oral route of infection occur by using same utensils (spoons, forks), toothbrushes, and kissing children mouth to mouth.

Fecal-oral route of infection occur by ingesting contaminated food or water due poor hygiene.

Present in the mucus that overlies the mucosa.

Helicobacter Pylori

The outcome of infection by H. Pylori reflects an interaction between {9}

- **Strain virulence** {10}
- Host Genotype
- Environmental factors

Genome {11}

1. Study of H pylori is centered on trying to understand the pathogenesis of genome database
2. H pylori consist of large diversity of strains with around 1,550 genes
3. H pylori contain 40kb-long Cag pathogenicity island (PAI) with over 40 pathogenic genes.
4. Asymptomatic patients carry H pylori strains lacking the Cag pathogenicity island (PAI).

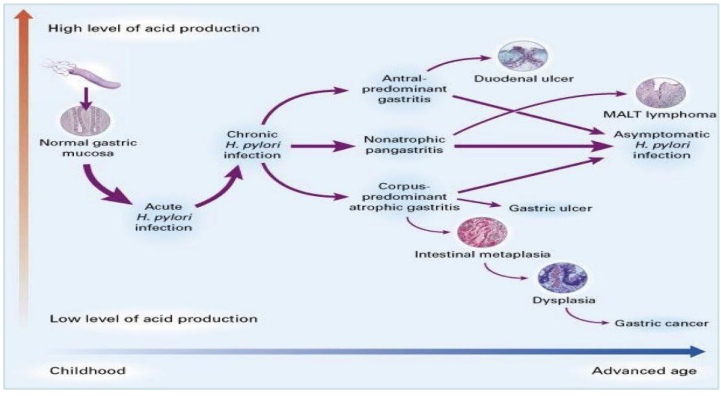
The whole slide is important

Pathophysiology of H Pylori

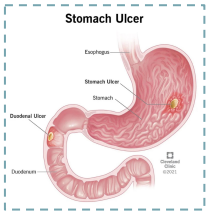
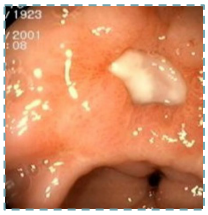
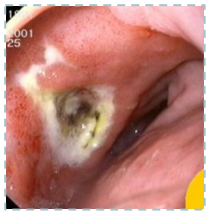
- To colonize the stomach, H pylori must survive acidity
- Using **flagella**, H pylori moves through stomach lumen and drill into the mucoid lining of stomach. **(motility)**
- Once it make contact with the stomach it will produces adhesions (outer membrane proteins) that binds to the epithelial cells
- Once H.pylori adhere to the stomach cells it will produces large amounts of **urease** enzyme that breaks down urea into CO2 + **ammonia**. This in-turn neutralizes gastric acid and helps it **survive the acidity.** {13}
- Ammonia is toxic to epithelial cells along with proteases**, vacA** protein (cause vacuole to the cell) and phospholipases produced by H. pylori and could damage epithelial cells. {14}
- Colonization of stomach or duodenum can result in chronic gastritis (inflammation of stomach lining) Inflammation stimulate more production of gastric acid
- This leads to gastric and duodenal ulcers, atrophy and later cancer.
- **CagA** (toxin associated with G-protein) protein was found to contribute to peptic ulcer and also cancer. (Remember: asymptomatic strains lack CagA), {12}
- Neutrophil-Activating Protein **(NAP)** recruits neutrophils to gastric mucosa causing inflammation. {15}
- Free radical production in the gastric lining due to H pylori, increases host cell mutation.
- H pylori induces the production of TNF-α and Interleukin 8 that leads to host cells mutation. {16}

Urease : H.pylori, It's very important in H.pylori survival
VacA protein : (vacuolating cytotoxin A) : Exotoxin causes apoptosis of cells
CagA protein :(cytotoxic associated protein A) : Exotoxin responsible for disrupting the cellular integrity and structure also promote inflammation
NAP :is a virulence factor of H. pylori that stimulates in neutrophils high production of oxygen radicals and adhesion to endothelial cells

The Notes from Dr of the graph is here. {17}



Peptic ulcer disease (PUD) {31}

Definition	Mucosal erosions (≥ 0.5cm) open sores that develop on the inside lining of your stomach and the upper portion of your small intestine the duodenum
Location	‣More peptic ulcers arise in duodenum than stomach. ‣Peptic ulcer is created in an acidic area very painful.
Characteristics	‣H. pylori infection is the main cause. ‣Associated with the over usage of NSAIDs, smocking, alcohol. {32}
Complications	‣Duodenal ulcers are generally benign. More common {33} ‣4% of stomach ulcer can turn to be malignant tumor.less common ‣Multiple biopsies are needed to exclude cancer.
Pictures	  Gastric ulcer  Duodenal ulcer

Signs and symptoms (of gastritis or ulcer)

Abdominal pain {34}, epigastric {35} with severity {36} relating to mealtime

Bloating and abdominal fullness.

Loss of appetite and weight loss.

Nausea and vomiting.

For both gastritis and ulcer

Haematemesis (vomiting of blood) due to gastric or esophagus damage.

Melena (blood in stool) foul-smelling & dark brown faeces due to oxidized hemoglobin iron. {37}

Rarely, Gastric or duodenal perforation leading to acute peritonitis (extremely painful-require urgent surgery). {38}

Only for ulcer

Helicobacter pylori

Description

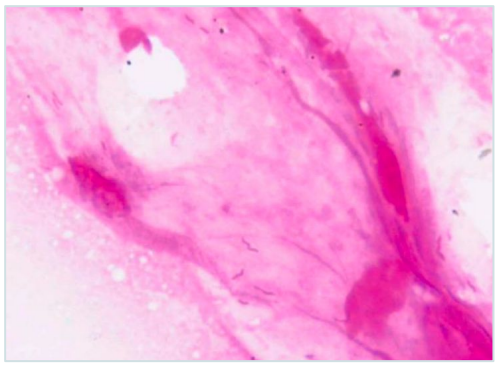
-Gram negative spiral bacillus
-Fastidious {18} in terms of growth requirements:
•Strictly microaerophilic (need low O2 to grow).
•Will grow in environments with increased Co2
•Blood agar based medium

-Hallmark of the species is production of urease enzyme :
• Urease breaks urea down to Co2+NH3
• Ammonia is a strong base
• Urease helps H. pylori survive strongly acidic stomach conditions.

-Very fragile -weak and easily die-
a point of importance when referring samples to the lab

Morphology & staining

Small, **Gram-negative**, **spiral rods**, **mobile** by **polar Flagella**.



Biochemistry

•Catalase-positive
•Oxidase- positive
•**Strongly urease-positive**.

Culture

• On blood agar based medium in a moist **microaerophilic atmosphere**.
• Selective medium can be used for isolation from clinical specimens
• Small colonies grow after 5-7 days at 37˚C.

Diagnosis

! Urea breath test !

Diagnosis
Checking dyspeptic patients for H. pylori

Here are the Dr's notes and summary of the diagnosis {24}

No -invasive methods
first line treatment (before invasive methods)

◦ **Serology** (Blood antibody) tests
poor accuracy : not that useful, poor accuracy. It doesn't correlate well to active signs and symptoms of the disease as the patient might have had positive antibodies for years after the infection..

◦ **Stool antigen test.** {19}

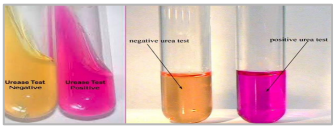
◦ **Carbon urea breath test (C14 or C13):** {20}
a urea solution labelled with C14 isotope is given to pt.
The Co2 subsequently exhaled by the pt contains the C14 isotope and this is measured. A high reading indicates presence of H. Pylori.

Invasive methods (most reliable), on biopsy:

◦ **Histological examination** of biopsy {21} specimens of gastric/duodenal mucosa take at endoscopy.
To check for ulcer or malignancy features

Gastric-biopsy specimen showing Helicobacter pylori adhering to gastric epithelium and underlying inflammation

◦ **Rapid urease test (CLO-test ®):** {22}
-based again on urease production by the organism → NH3 production → rise in pH → change in the colour indicator of the kit
- High sensitivity & specificity
- Prompt result.



Rapid urease test A biopsy of mucosa is taken from antrum of the stomach, and is placed into a medium containing urea and indicator such as phenol red. The urease produced by H. pylori hydrolyzes urea to ammonia, which raises the pH of the medium, and changes the color of the specimen from yellow (-ve) to red (+ve)

◦ **Culturing** the bacteria Used for antibiotic resistance testing , as sensitive as the histology. Requires selective agars and incubation for growth. it needs special media . H.Pylori is a very difficult organism to culture because the it is very fragile (dies quickly) and fastidious, so a special transport media is needed. However; **it might be helpful in relapse patients to detect antimicrobial sensitivity and resistance and failure of treatment.**

◦ **Molecular methods** (e.g. PCR) {23}

Susceptibility Testing

◦ Not available in all centers
◦ Require growth from culture, so biopsy needed
◦ More recently molecular methods looking for mutations that code for resistance have been used

Management of Helicobacter pylori

Prevention	◦ Treatment and eradication of infection will - **Improve symptoms** Such as (dyspepsia, gastritis, peptic ulcer and cancer) -Potentially **reverse progression** ◦ Vaccination: - Promising results with newer formulations - No vaccine available yet ◦ Dietary methods: {25} - eating broccoli, cabbage, honey, and drinking green tea. ◦ Proper sanitation and clean sources of drinking water and good hygiene standard.
Antibiotic sensitivity {26}	-In vitro (inside the lab) H.pylori is sensitive to amoxicillin, tetracycline, metronidazole, macrolides (clarithromycin). - in vivo (inside the human body) their efficacy is often poor due to the low pH of the stomach, their failure to penetrate the gastric mucus and the low concentration of antibiotic obtained in the mucosa of the stomach. -Recently , Metronidazole in developing countries is becoming resistance (80-90%).
Treatment Regimens {27}.	Different regimens available : ‣Clarithromycin triple therapy: {28} -PPI b.d. (twice a day) + clarithromycin + amoxicillin or metronidazole for 14 days ‣Bismuth quadruple therapy (first line therapy): {29} - Proton pump inhibitor (PPI) + bismuth subsalicylate/subcitrate +metronidazole + tetracycline for 14 days
Post Treatment Testing	After identification and treatment, eradication should be proven using: {30} – Urea breath test – Fecal antigen test – Biopsy based testing (in high risk).

Dr Notes

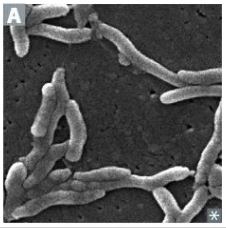
{1} Why is H. pylori important? Because it's oncogenic (promotes cancer) specifically carcinoma (directly linking to causing cancer).
{2} It only exists in humans (only human to human transmission).
{3} In case it symptomatic, what is the symptoms? Acute gastritis, chronic gastritis, ulcer, cancer (if not treatment) such as adenocarcinoma or lymphoma.
{4} H.pylori spread in developing countries more than developed countries because developing countries have less hygiene and they always share things, so bacteria spread between them easily.
{5} The most 80% is asymptomatic but in case symptomatic is 20%.
{6} Ulcer It happens anywhere but most often in proximal duodenum and antrum area of stomach.
{7} Organisms that cause (promot) cancer called oncogenic, and we can divide it into two classification: 1-virus: like EBV, HPV, hepatitis. 2-Bacteria: like H.pylori.
{8}general notes :1-Some people get gastritis and others get gastritis with ulcer, others get ulcer then develop to cancer.
2- The difference between infection and disease is that infection is You are infected whether you have symptoms or not, but disease is always symptomatic.
3-If the person does not have a symptoms he called "colonization".
{9} What causes some people to have strong symptoms and others not?
1-Genes and a person's immunity 2-risk factors 3-smoking and alcohol consumption.
{10} If we have patient with H.pylori and does not have the essential virulence factors this will not cause disease or maybe cause mild disease.
{11} Absence them (like cag, vac) leads to less sever, a lot of virulence factor H.pylori.
{12} Most H.pylori have flagella and urease, but not all have Cag and Vac.
{13} The problem about ammonia in two things: 1-It is alkaline and protects H.pylori from stomach acid. 2-It is considered toxic for gastro cell, and without ammonia the H.pylori can not live long time due to gastric acid.
{14} VacA protein (vacuolating cytotoxin A): exotoxin causes apoptosis of cells.
{15} More inflammation mean more gastritis which lead to destruction of cell.
{16} the TNF-α and IL-8 produced by the immune system.
{17}1- At each stage of infection the amount of gastric acid will vary (we do not talk in these in details). 2- H.pylori that has no Cag and Vag doesn't cause very severe diseases (asymptomatic). 3-what is the general virulence factors of H.pylori? A-outer membrane B-urease. C-proteins and phospholipase. D-Vac and Cag (very important to cause disease).
{18} Fastidious bacteria need special nutritional supplements so they can grow.
{19} If the result of stool antigen and urease test is positive that means the patient has active disease cause H.pylori.But in Serology tests, if the result is positive, it does not mean that the patient has an active H. pylori infection
{20} This test mean that patient drink urea solution (which contain labelled or C14) then if his stomach has H.pylori the urea breakdown due to urease which is formation CO2 the patient will exhale it, then put it in device and look.

Dr Notes

{21} It need endoscope to see if there is ulcer or not, if it ulcer they will take a sample (to check if there is cancer thanges or not).
{22} Take the sample then put it in urea and then the result it will be positive.
{23} If we do culturing and molecular that means take sample (biopsy) from ulcer.
{24} The diagnosis of the patient depend on situation of him, if it was initially diagnosed do stool antigen test, but if it doubt he has ulcer or recurrent disease do the scope which means take sample (biopsy) from ulcer and doing pathological examination and culture if available.
{25} Some patient prefer certain foods because it relax them, which varies from one patient to another.
[26} There are many obstacles to antibiotics, such as stomach acid, as they do not work in that environment.
{27} What is the principle of treatment? Use several medications and the regimens contain anti acidic and antibiotic.
{28} But it is less used because resistance to some of the options in it has increased.
{29} What is the benefit of anti acidic? 1-for the antibiotic to work. 2-the acid environment cause pain for the patient so antibiotic treat this symptom.
{30} After four weeks of this course doing testing to follow up and see if the infection stop or not.
{31} The peptic ulcer is a general name of gastric ulcer and duodenal ulcer.
{32} The peptic ulcer causes by H.pylori but there is other risk factors like NSAIDS, smoking and alcohol.
{33} possibility that it will develop to cancer (malignant) less than stomach.
{34} Depend if it acute or chronic.
{35} The location of pain is epigastric area. the description of the pian is burning, dull.
{36} The time of pain various depend if it gastric or ulcer, but in general it was sever if the stomach empty.
{37} Melena means bleeding in upper GIT, then pass in all GIT tract and appear dark due to oxidation (causes ulcer in stomach and duodenum).
{38} if the patient has ulcer and not treat it that will cause perfusion in wall of stomach then he need to surgery (emergency situation), also the infection will happen which is (peritonitis) due to exposure the GI content to peritoneum cavity which is sterile environment.

Extra: First Aid summary

Helicobacter pylori



Curved, flagellated (motile), gram ⊖ rod A that is **triple** ⊕: catalase ⊕, oxidase ⊕, and urease ⊕ (can use urea breath test or fecal antigen test for diagnosis). Urease produces ammonia, creating an alkaline environment, which helps *H pylori* survive in acidic mucosa. Colonizes mainly antrum of stomach; causes gastritis and peptic ulcers (especially duodenal). Risk factor for peptic ulcer disease, gastric adenocarcinoma, and MALT lymphoma.

Summary

Extra

Diseases caused by H pylori	1. Chronic or acute active gastritis 2. Gastric & Duodenal ulcer (Peptic ulcer) 3. Gastric adenocarcinoma 4. Gastric mucosa-associated lymphoid tissue (MALT) lymphoma
Transmission	○ Contagious with an **unknown** route of transmission. ○ Person to person (oral to oral or fecal-oral) route. ○ Gastric antrum of the stomach is the most favoured site ○ Increases oral-oral route of infection occur by kissing ○ Fecal-oral route of infection occur by ingesting contaminated Food or water due poor hygiene.
Outcome	**The outcome of infection by H. Pylori reflects an interaction between:** 1. Strain virulence 2. Host Genotype 3. Environmental factors **Note** :Asymptomatic patients carry H pylori strains lacking the Cag pathogenicity island (PAI).
Pathogenesis	○ Using **flagella**, H pylori moves through stomach lumen and drill into the mucoid lining of stomach. ○ Once it make contact with the stomach it will produces adhesions (outer membrane proteins) that binds to the epithelial cells ○ Once H.pylori adhere to the stomach cells it will produces large amounts of **urease** enzyme that breaks down urea into CO2 + **ammonia**. This in-turn neutralizes gastric acid and helps it **survive the acidity.** ○ Ammonia is toxic to epithelial cells along with proteases**, vacA** protein (cause vacuole to the cell) and phospholipases produced by H. pylori and could damage epithelial cells. ○ Colonization of stomach or duodenum can result in chronic gastritis (inflammation of stomach lining) Inflammation stimulate more production of gastric acid ○ This leads to gastric and duodenal ulcers, atrophy and later cancer. ○ **CagA** protein was found to contribute to peptic ulcer and also cancer. ○ Neutrophil-Activating Protein **(NAP)** recruits neutrophils to gastric mucosa causing inflammation. ○ Free radical production in the gastric lining due to H pylori, increases host cell mutation. ○ H pylori induces the production of TNF-α and Interleukin 8 that leads to host cells mutation.

Peptic ulcer disease (PUD)

Definition	Mucosal erosions (≥ 0.5cm)
Location	More peptic ulcers arise in duodenum than stomach.
Characteristics	‣ H. pylori infection is the main cause. ‣ Associated with the over usage of NSAIDs, smocking, alcohol.
Complications	‣ Duodenal ulcers are generally benign ‣ 4% of stomach ulcer can turn to be malignant tumor
Symptoms	1. Abdominal pain, epigastric with severity relating to mealtime 2. Bloating and abdominal fullness. 3. Loss of appetite and weight loss. 4. Nausea and vomiting. 5. Haematemesis (vomiting of blood) due to gastric or esophagus damage. 6. Melena foul-smelling & dark brown faeces due to oxidized hemoglobin iron. 7. Rarely, Gastric or duodenal perforation leading to acute peritonitis (extremely painful require urgent surgery).

Summary

Extra

H pylori

Description	◎ Fastidious in terms of growth requirements: ○ Strictly microaerophilic (need low O2 to grow). ○ Will grow in environments with increased Co2 ○ Blood agar based medium ◎ Hallmark of the species is production of urease enzyme : ○ Urease breaks urea down to Co2+NH3 ○ Ammonia is a strong base ○ Urease helps H. pylori survive strongly acidic stomach conditions. ◎ Very fragile -a point of importance when referring samples to the lab-	
Morphology & staining	Small, Gram-negative, spiral rods, mobile by polar Flagella.	
Culture	○ On blood agar based medium in a moist microaerophilic atmosphere. ○ Selective medium can be used for isolation from clinical specimens ○ Small colonies grow after 5-7 days at 37˚C.	
Biochemistry	○ Catalase-positive ○ Oxidase- positive ○ Strongly urease-positive.	
Diagnosis	**No -invasive methods**	‣ Serology : poor accuracy ‣ Stool antigen test ‣ Carbon urea breath test (C14 or C13
	Invasive methods	‣ Rapid urease test (CLO-test ®) ‣ Histological examination of biopsy ‣ Molecular methods ‣ Culturing the bacteria Used for antibiotic resistance testing

Management

Prevention	○ Treatment and eradication of infection will : ↠ **Improve symptoms** ↠ Potentially **reverse progression** ○ Vaccination: No vaccine available yet ○ Dietary methods ○ Proper sanitation and clean sources of drinking water and good hygiene standard.
Antibiotic sensitivity	○ In vitro ○ in vivo (inside the human body) their efficacy is often poor due to the low pH of the stomach, their failure to penetrate the gastric mucus and the low concentration of antibiotic ○ Recently , Metronidazole in developing countries is becoming resistance
Treatment Regimens	Different regimens available: ‣ Bismuth quadruple therapy (first line therapy): Proton pump inhibitor (PPI) + bismuth subsalicylate/subcitrate +metronidazole + tetracycline for 14 days
Post Treatment Testing	After identification and treatment, eradication should be proven using: – Urea breath test – Fecal antigen test – Biopsy based testing

MCQs

Q1 - All the following are features H. Pylori organism EXCEPT:			
A) Urease +	B) Gram +	C)Microaerophilic	D) Spiral bacilli
Q2 - H. Pylori is considered a risk factor for:			
A) Gastric adenocarcinoma	B) MALT lymphoma	C) Peptic ulcer	D) All the previous
Q3 - Which of the following can cause damage to the epithelial cells ?			
A) Flagella	B) Proteases	C) Ammonia	D) B+C
Q4 - Which of the following is an invasive method for diagnosis of H. Pylori ?			
A) Histological exam of biopsy	B) Stool antigen test	C) Carbon urea breath test	D) Serology
Q5 - All the following are signs for Peptic ulcer EXCEPT:			
A) Nausea & vomiting	B) Haematemesis	C) Epigastric burning	D) Weight gain

Answer Key: 1-B 2-D 3-D 4-A 5-D

SAQ

Case

a 40 years old women presented to family medicine clinic with 1 month history of epigastric pain, burning in nature, more severe on empty stomach. Recently, patient started to develop nausea and vomiting of blood as well.

Q1: What is the most likely diagnosis?
Q2: What is the most likely causative agent?
Q3: What are the virulence factors possessed by this organism?
Q4: What are the diagnostic methods used to detect this organism?
Q5: What is the spectrum of diseases that are usually caused by this organism?
Q6: What is the suggested initial treatment regimen?

Answers
A1: Peptic Ulcer
A2: Helicobacter Pylori
A3 :1. Urease (Breakdown urea into CO2 and ammonia which will neutralize gastric acid and cause damage to tissue (by ammonium which is toxic to tissue)
2. CagA (Cytokine release (IL-8), Has a role in the development of cancer) 3. VacA (Gastric tissue damage)
A4: 1. Invasive methods such as: Rapid urease test, Culture of bacteria or histology if we suspect cancer.
2. Non invasive methods such as: Stool antigen test, Carbon urea breath test
A5: Chronic active gastritis - Gastric and duodenal ulcer (peptic ulcer) - Gastric adenocarcinoma - Gastric mucosa associated lymphoid tissue (MALT) lymphoma
A6: Proton pump inhibitor (PPI) + bismuth subsalicylate/subcitrate +metronidazole + tetracycline for 14 days

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