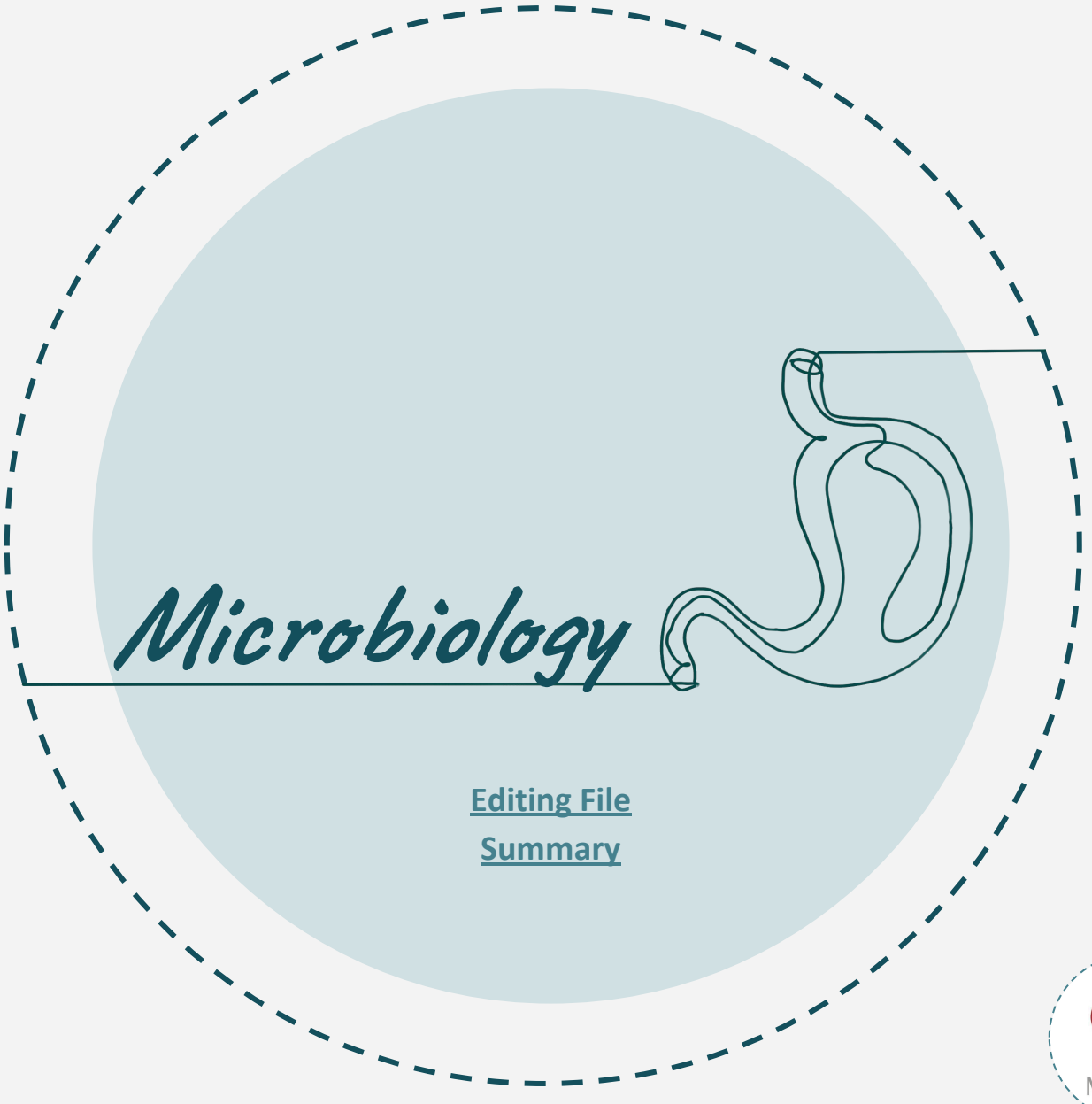
Helicobacter Pylori

(جرثومة المعدة)



Editing File

Summary

MED439
KING SAUD UNIVERSITY

MICROBIOLOGY
Team 439

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Objectives:

- Explain the various gastric and duodenal diseases caused by H.pylori.
- Discuss the epidemiology and transmission of H. pylori.
- 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.
- Discuss preventative methods used for H. pylori infection.
- Describe the management and treatment regiments used for eradication of H. pylori.

Helicobacter Pylori

General info

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.
There is no evidence of animal-to-human transmission

Epidemiology

Around 50% of world's population harbor H pylori.[1]

Over 80% of individuals infected with the bacterium are asymptomatic

H. Pylori is a bacteria that is common worldwide, and it can cause a wide spectrum of diseases. Most patients will be asymptomatic carriers (have infection but not the disease). However, other patients might develop the disease with different severities

Third world and developing countries have more rate of infection.

Infections are usually acquired at childhood.

Poor sanitary conditions contribute to high rates.

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

Prevalence varies greatly among countries and population groups

Overall frequency of H pylori infection is declining.

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

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

[1] 50% of people have H.pylori living inside them " Commensal Bacteria "

Helicobacter Pylori

Helicobacter pylori is found closely associated with gastric mucosa and is an independent risk factor for the development of:	
1	chronic active gastritis
2	gastric & duodenal ulcer (Peptic ulcer)
3	Gastric adenocarcinoma
4	Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

Transmission

1. Contagious with an **unknown** route of transmission.
2. **Person to person** (oral to oral or fecal-oral) route.
3. **Transmission** occur mainly within families or community.
4. **Gastric antrum** is the most favoured site of colonization.
   Usually, H.pylori colonizes the antrum of the stomach, but other areas can be colonized as well.
5. **Oral-oral route** of infection occur by using same utensils (spoons, forks), toothbrushes, and kissing children mouth to mouth.
6. **Fecal-oral route**[1] of infection occur by ingesting contaminated food or water due poor hygiene.
7. Present in the mucus that overlies the mucosa.

[1] A particular route of transmission of a disease wherein pathogens in fecal particles pass from one person to the mouth of another person , the main causes of fecal-oral transmission are:

- lack of adequate sanitation and poor hygiene
- Contaminated food due to lack adequate sanitation

Helicobacter Pylori

The outcome of infection by H. Pylori reflects an interaction between [1]

- Strain virulence
- Host Genotype
- Environmental factors

Genome

H pylori consist of large diversity of strains with around 1,550 genes

Study of H pylori is centered on trying to understand the pathogenesis of genome database

H pylori contain 40kb-long Cag pathogenicity island (PAI) with over 40 pathogenic genes.

Asymptomatic patients carry H pylori strains **lacking** the Cag pathogenicity island (PAI).

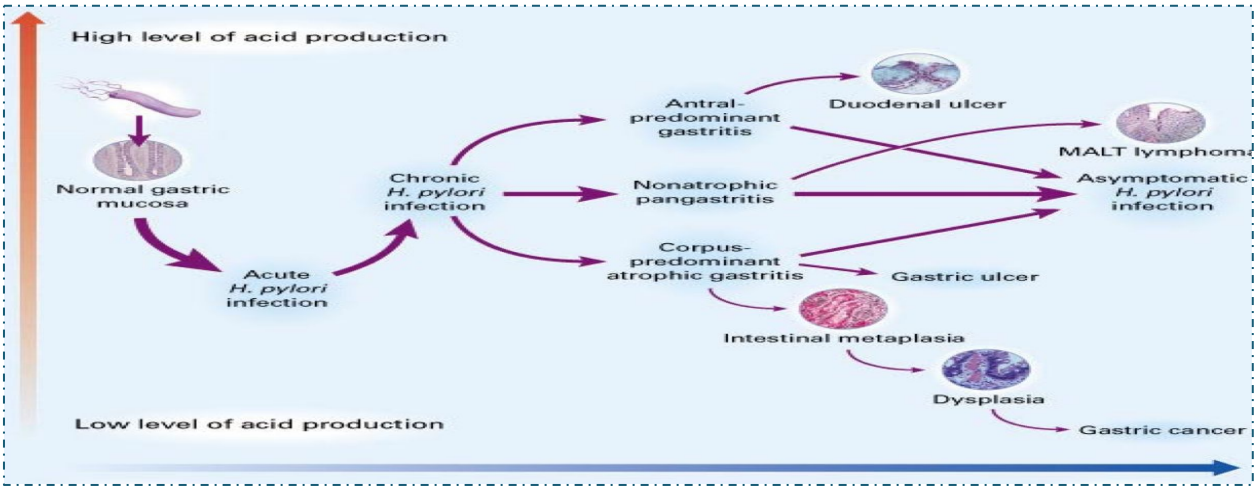
[1] Outcome, severity, and symptoms depend on many factors:
(1) Strain virulence: H. Pylori has different strains each possessing different virulence factors.
(2) Host genotype and immunity.
(3) Environmental factors that lead to exposure to the bacteria such as low socioeconomic standards, poor hygiene, and water pollution in 3rd world countries.

Strain virulence:
Urease: helps to break urea into Co2 and ammonia (ammonia is alkaline so it decreases the acidity of stomach and helps H.pylori survive stomach acidity. In addition, ammonia itself is toxic to the epithelial cells).
Flagella: help in movement (motility).
VacA: vacuolating cytotoxin that breaks cells down (pore forming).
CagA: cytotoxin with high oncologic potential (strongest risk factor for gastric cancer). When it is injected into cells, it will start a cascade of cellular changes that leads to ulcers and potentially cancer (along with other previously mentioned risk factors). Strains that lack CagA usually do not cause significant disease (asymptomatic).

Helicobacter Pylori

Pathogenesis

- To colonize the stomach, H pylori must survive acidity
- Using **flagella**[1], 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**[2] enzyme that breaks down urea into CO2 +ammonia. This in-turn neutralizes gastric acid and helps it survive the acidity (by Ammonia)
- Ammonia is toxic to epithelial cells along with proteases, **vacA**[3] protein 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**[4] protein was found to contribute to peptic ulcer. (Remember:asymptomatic strains lack CagA)
- Neutrophil-Activating Protein **(NAP)**[5] 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



(1)Help in motility (H.pylori is able to move down towards the cells of the stomach with the help of its flagella)

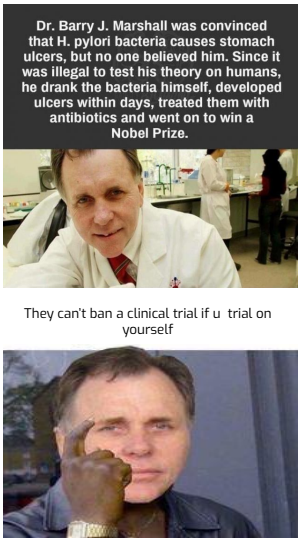
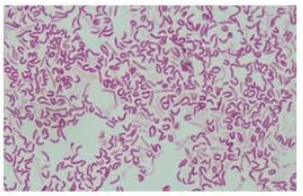
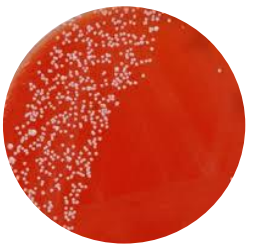
(2)H.pylori, It's very important in H.pylori's survival

(3)VacA protein (vacuolating cytotoxin A) : Exotoxin causes apoptosis of cells

(4)CagA protein (cytotoxic associated protein A) : Exotoxin responsible for disrupting the cellular integrity and structure also promote inflammation

(5)is a virulence factor of H. pylori that stimulates in neutrophils high production of oxygen radicals and adhesion to endothelial cells

Helicobacter Pylori

Description	-Fastidious in terms of growth requirements: ○ Strictly **microaerophilic** ○ Will grow in environments with increased CO_2 ○ Blood agar based medium. -Hallmark of the species is production of urease enzyme: ○ Urease breaks urea down to CO_2 + NH_3 ○ 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	Small, **Gram-negative**, **spiral bacilli** (rods), motile by polar flagella	
Culture	○ On blood agar based medium in moist microaerophilic atmosphere ○ Selective medium can be used for isolation from clinical specimens ○ Small colonies grow after 5-7 days at 37℃ (very slow)	
Biochemistry	○ Catalase positive ○ Oxidase positive ★ **Strongly urease positive**	

Helicobacter Pylori

★★★ Diagnosis

Checking **dyspeptic patients** for *H. pylori*:

★ **Non-Invasive methods**

- **Serology** -blood antibody- test (poor accuracy)
  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 (more helpful in epidemiological studies).
- **Stool antigen test** detects H. pylori antigen in feces.

Carbon urea breath test (C^{14} or C^{13}) (best non-invasive option)
A urea solution labelled with C^{14} isotope is given to pt. The CO_2 subsequently exhaled by the pt contains the C^{14} isotope and this is measured. A high reading indicates presence of *H. Pylori*

It is the test usually done in KKUH (under radiology department). Procedure: patient swallow urea labelled with radioactive carbon-14. In the subsequent 10–30 minutes, the detection of labelled carbon dioxide in exhaled breath indicates that the urea was split; this indicates that urease (the enzyme that H. pylori uses to metabolize urea) is present in the stomach, and hence that H. pylori is present.

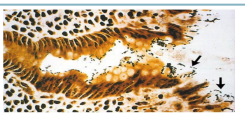
★ **Invasive methods (most reliable), on biopsy:**

- **Histological examination** of biopsy specimens of gastric/duodenal mucosa take at endoscopy.
- **Rapid urease test (CLO-Test ®):** A biopsy of mucosa is taken from the antrum of the stomach, and is placed into a medium containing urea and an 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)

  - High sensitivity and specificity
  - Prompt result
  - **Based again on:**
    1-Urease production by the organism
    2-NH_3 is Produced
    3-Rise in pH
    4-Change in color of kit

- **Culturing** the bacteria. Used for **antibiotic resistance** testing, as sensitive as the histology. Requires selective agars and incubation for growth.
  it needs special media such as Marshall's Brain Heart Infusion, brucella agar, or columbia agar. H.Pylori is a very difficult organism to culture because the it is very fragile and fastidious (dies quickly), so a special transport media is needed. However; it might be helpful in relapse patients to detect antimicrobial sensitivity and resistance.
- **Molecular** methods (e.g. PCR)

- Gastric biopsy specimen showing *Helicobacter Pylori* adhering to gastric epithelium and underlying inflammation
  Note that the bacteria is embedding itself into mucus lining (hiding from stomach acidity)



Treatment

Dr. khalifa:
لو تذكرتوا وحده فيهم زي
Clarithromycin triple therapy
It should be enough
(details about dose and time etc not imp)

Treatment and eradication of infection will improve symptoms (dyspepsia, Gastritis, Peptic ulcer and cancer) and can potentially reverse progression.

- **Prevention:**
  - **Vaccination** No vaccine available yet but there are promising results with newer formulations.
  - **Dietary methods**: eating broccoli, cabbage, honey, and drinking green tea.
  - **Proper sanitation and clean sources of water**
- **Antibiotic sensitivity**
  - **In Vitro:** *H. Pylori* is sensitive to amoxicillin, tetracycline, metronidazole, macrolides (clarithromycin)
  - **In Vivo:** Antibiotic efficacy is 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.
  - **Metronidazole is becoming resistant in developing countries**
- **Treatment Regimens:**
  - **Clarithromycin triple therapy:-**
    Proton- Pump inhibitor (twice a day) + clarithromycin + amoxicillin or metronidazole[1] for 14 days.
  - **Bismuth quadruple therapy:-**
    - Proton- Pump inhibitor + Bismuth subsalicylate/Subcitrate + Metronidazole + tetracycline for 10-14 days
    - Used as Salvage therapy if triple therapy fails
    - Another salvage option: Levofloxacin + Amoxicillin + PPI

After Identification and treatment, Eradication should be proven using:
- Urea breath test
- Fecal antigen test
- Biopsy based testing

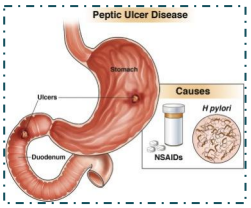
Susceptibility testing

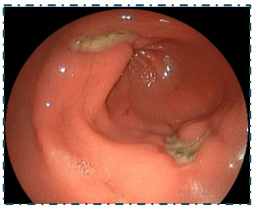
- Not available in all centers
- Require growth form culture, so biopsy needed
- More recently molecular methods looking for mutations that code for resistance
- have been used

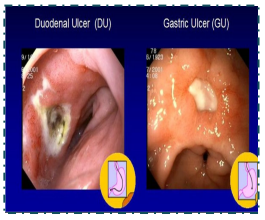
[1] Also known as Flagyl
a) Used against anaerobes & parasites.
b) Acts by interacting with DNA and causing strand breakage.
c) Belongs to nitroimidazole class of antibiotics

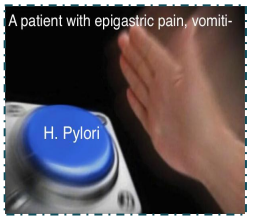
Peptic Ulcer

Definition	Mucosal erosions (≥0.5 cm)
Location	○ More Peptic ulcers arise in the **duodenum** than the stomach ○ Peptic ulcers arise in an acidic area, thus they are very painful
Characteristics	○ Associated with the overuse of **NSAIDS, smoking, alcohol** ○ Duodenal ulcers are usually **benign** ○ H. Pylori is the main cause
Complications	4% of stomach ulcer can turn to be **malignant** tumor. (Multiple biopsies are needed to exclude cancer)









★ Signs and symptoms

Abdominal epigastric **burning in nature** with severity relating to mealtime[1] 3 hours after meal with gastric ulcer

Bloating and abdominal fullness

Nausea and vomiting

Haematemesis
Vomiting of blood due to gastric or esophageal damage

Melena Foul-smelling and dark brown faeces due to oxidized hemoglobin iron

Rarely, Gastric or duodenal perforation leading to acute peritonitis Extremely painful-require urgent surgery

Loss of appetite and weight loss

[1] Patient with ulcers mainly present to the GP or family medicine clinic with **epigastric pain that is burning in nature, and has been consistent for several weeks.** If treated properly, the progression of the disease can be reversed.

Drs' notes

Dr. Khalifa

- H. Pylori is a bacteria that is common worldwide, and it can cause a wide spectrum of diseases. Most patients will be asymptomatic carriers (have infection but not the disease). However, other patients might develop the disease with different severities; some will have gastritis while others will **develop duodenal/gastric ulcers that have the potential to turn into cancer (adenocarcinoma/lymphoma)**.
- Usually, H.pylori colonizes the antrum of the stomach, but other areas can be colonized as well.
- ★ Patient with ulcers mainly present to the GP or family medicine clinic with **epigastric pain that is burning in nature, and has been consistent for several weeks.** If treated properly, the progression of the disease can be reversed.
- Outcome, severity, and symptoms depend on many factors: (1) Strain virulence: H. Pylori has different strains each possessing different virulence factors. (2) Host genotype and immunity. (3) Environmental factors that lead to exposure to the bacteria such as low socioeconomic standards, poor hygiene, and water pollution in 3rd world countries.
- ★ **Strain virulence:**
  - **Urease:** helps to break urea into Co2 and ammonia (ammonia is alkaline so it decreases the acidity of stomach and helps H.pylori survive stomach acidity. In addition, ammonia itself is toxic to the epithelial cells).
  - **Flagella:** help in movement (motility).
  - **VacA:** vacuolating cytotoxin that breaks cells down (pore forming).
  - **CagA:** cytotoxin with high oncologic potential (strongest risk factor for gastric cancer). When it is injected into cells, it will start a cascade of cellular changes that leads to ulcers and potentially cancer (along with other previously mentioned risk factors). Strains that lack CagA usually do not cause significant disease (asymptomatic).
- NOT IMPORTANT: pH in stomach varies depending on the stage. In beginning it will be high. As bacteria produces urease and other factors, pH of gastric acids will increase. (More destruction = less gastric acidity).
- **Ulcers are more painful when stomach is empty** as there is nothing to buffer the acid (increased irritability to the ulcerated area).
- Stomach ulcer is generally more worrying than duodenal ulcer because it has a higher chance to form a cancer.
- When a patient is suspected to have H. Pylori, we start with non invasive methods and treatment is given accordingly. If the initial treatment fails (patient relapsed/resistance) or if there is a suspicion of ulcer/cancer, invasive methods that involve taking mucosal biopsy must be done.
- ★ **Non invasive diagnostic methods:**
  - **Carbon urea breath test:** It is the test usually done in KKUH (under radiology department). Procedure: patient swallow urea labelled with radioactive carbon-14. In the subsequent 10–30 minutes, the detection of labelled carbon dioxide in exhaled breath indicates that the urea was split; this indicates that urease (the enzyme that H. pylori uses to metabolize urea) is present in the stomach, and hence that H. pylori is present.
  - **Stool immunoassay test:** detects H. pylori antigen in feces.
  - **Serology:** 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 (more helpful in epidemiological studies).
  - **Molecular methods:** usually for research purposes.
- ★ **Invasive diagnostic methods:**
  - **Histochemical examination.**
  - **Culturing of H. Pylori:** it needs special media such as Marshall's Brain Heart Infusion, brucella agar, or columbia agar. H.Pylori is a very difficult organism to culture because the it is very fragile and fastidious (dies quickly), so a special transport media is needed. However; it might be helpful in relapse patients to detect antimicrobial sensitivity and resistance.
  - **Rapid urease test:** A biopsy of mucosa is taken from the antrum of the stomach, and is placed into a medium containing urea and an 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).
- Treatment requires 3-4 medications, at least two of which need to be antibiotics. A multidrug regimen is required because the drugs are unable to penetrate the gastric and duodenal mucosa (due to bacterial colonization). The choice of drugs depends on many factors including prior failed therapy, allergies, and recent use of antibiotics.

Dr. Fawzia

- H. pylori is the most prevalent infection in the world.
- Upon entry of bacteria to the stomach, the bacteria will use its flagella to help it move and drill into the mucoid lining (to avoid acidity). Then, it will produce a membrane protein to help it adhese. Also, it will produce urease to further protect itself from acidity of the mucosa.
- It is closely related to anaerobes, but it can tolerate a little amount of oxygen.
- Serology is done by looking for antigens in the blood but it is has poor accuracy.

Quiz

MCQ

Q1: Which of the following is NOT a symptom of peptic ulcer?

A- Nausea
B- Weight loss
C- Retrosternal burning sensation
D- Melena

Q2: A patient is suspected to have peptic ulcer, which of the following is the most reliable diagnostic approaches?

A- Rapid Urease test
B- Serology
C- Carbon Urea Breath test
D- Stool Antigen test

Q3: Which of the following is a virulence factor of H.pylori that produces ammonia and neutralizes stomach acidity?

A- CagA
B- VacA
C- Adhesions
D- Urease

Q4: Which of the following is the appearance of *Helicobacter Pylori* under the microscope?

A- Gram +ve Cocci in pairs
B- Gram +ve Spiral Bacilli
C- Gram -ve Cocci in pairs
D- Gram -ve Spiral Bacilli

Q5: Which of the following is the Biochemistry test results for *Helicobacter Pylori* ?

A- Urease +ve, Catalase -ve, Oxidase +ve
B- Urease +ve , Catalase -ve, Oxidase +ve
C- Urease +ve, Catalase +ve, Oxidase +ve
D- Urease -ve, Catalase +ve, Oxidase -ve

Q6: A 46-year-old man presents with a 3-week history of burning substernal and epigastric pain that improves after meals. Medical history is significant for hypertension, which is controlled with exercise and diet. Medications Include a daily vitamin supplement. He has no other complaints. social history reveals that the patient was born in Cambodia, and lived there until 5 years ago when he moved to the United States. A urease breath test performed in the office is positive. What is the recommended treatment?

A- Ampicillin, clindamycin, and bismuth
B- Omeprazole, clarithromycin, and metronidazole or amoxicillin
C- Ranitidine, omeprazole, and bismuth
D- Vancomycin, gentamicin, and aztreonam

Answers: Q1:C | Q2:A | Q3:D | Q4:D | Q5:C| Q6:B

SAQ

Dr.Khalifa: 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?

A: Peptic Ulcer

Q2: What is the most likely causative agent?

A: Helicobacter Pylori

Q3: What are the virulence factors possessed by this organism? (Extra)

A: 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)

H.pylori: *Exists*

Peptic ulcer:

Q4: What are the diagnostic methods used to detect this organism? (Extra)

A: 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

Q5: What is the spectrum of diseases that are usually caused by this organism? (Extra)

A: Chronic active gastritis - Gastric and duodenal ulcer (peptic ulcer) - Gastric adenocarcinoma - Gastric mucosa associated lymphoid tissue (MALT) lymphoma

Q6: What is the suggested initial treatment regimen? (Extra)

A: Clarithromycin triple therapy:- Proton- Pump inhibitor (twice a day) + clarithromycin + amoxicillin or metronidazole for 14 days

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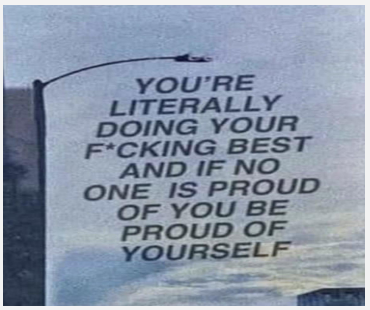

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