AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 4.

Microbiology diagnosis of gastro-intestinal bacterial diseases

(dysentery, cholera, campylobacteriosis, helicobacteriosis)

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. Shigella, morpho-biological characteristics.

• Pathogenesis and clinical manifestations of bacterial dysentery

• Microbiological diagnosis of bacterial dysentery, determination of bacterial carriage.

• Specific treatment and prevention of bacterial dysentery.

• Mechanism of resistance to extended-spectrum beta-lactamase (ESBL).

2. General properties, classification, biovars and serovars of vibrios

• Morpho-biological characteristics of cholera vibrios.

• Differentiation of cholerae, El-Tor biovars and vibrios from serogroup O139.

• Pathogenesis and clinical manifestations of cholera

• Microbiological diagnosis of cholera.

• Specific treatment and prevention of cholera

3. Morpho-biological characteristics of Campylobacter, pathogenesis and clinical manifestations of diseases caused by it

• Microbiological diagnosis of campylobacteriosis

4. Helicobacter pylori, morpho-biological characteristics, pathogenicity factors, role in the pathogenesis of gastritis, gastric and duodenal ulcer, gastric cancer and MALT-lymphoma

• Microbiological diagnosis of helicobacteriosis, application of invasive and non-invasive examination methods. Urease breath test (UBT)

Purpose of the lesson:

- To acquaint students with the morpho-biological characteristics of the causative agents of dysentery, cholera, campylobacteriosis and helicobacteriosis, to teach the pathogenesis, clinical signs, microbiological diagnostic methods, specific treatment and prevention principles of the diseases caused by them.

Shigella -Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Gammaproteobacteria
- (Order): Enterobacterales
- (Family): Enterobacteriaceae
- (Genus): ***Shigella***
- **(Species):** ***S.dysenteriae, S.flexneri, S.boydii, S.sonnei***

CLASSIFICATION

- 4 SPECIES/SUBGROUPS BASED ON BIOCHEMICAL AND SEROLOGICAL CHARACTERS
- SHIGELLA DYSENTERIAE : 12 Serotypes
- SHIGELLA FLEXNERI : 6 serotypes
- SHIGELLA BOYDII : 18
- SHIGELLA SONNEI : 17 Colicins types

Taxonomy

Family *Enterobacteriaceae*

1. ***Shigella dysenteriae***: most serious form of bacillary dysentery
2. ***Shigella flexneri***: shigellosis in underdeveloped countries
3. ***Shigella sonnei***: shigellosis in developed countries
4. ***Shigella boydii***

Morphology & Physiology

- Small Gram-negative, facultatively anaerobic, coliform bacillus
- Non-motile (no H antigen)
- Possess capsule (K antigen) and O antigen
- **K antigen** not useful in serologic typing, but can interfere with O antigen determination
- **O antigens**: A, B, C, D correspond respectively to the four species
- Non-lactose fermenting
- Bile salts resistant: trait useful for selective media

ferment glucose

Reduce nitrates (NO_3 to NO_2 or N_2)

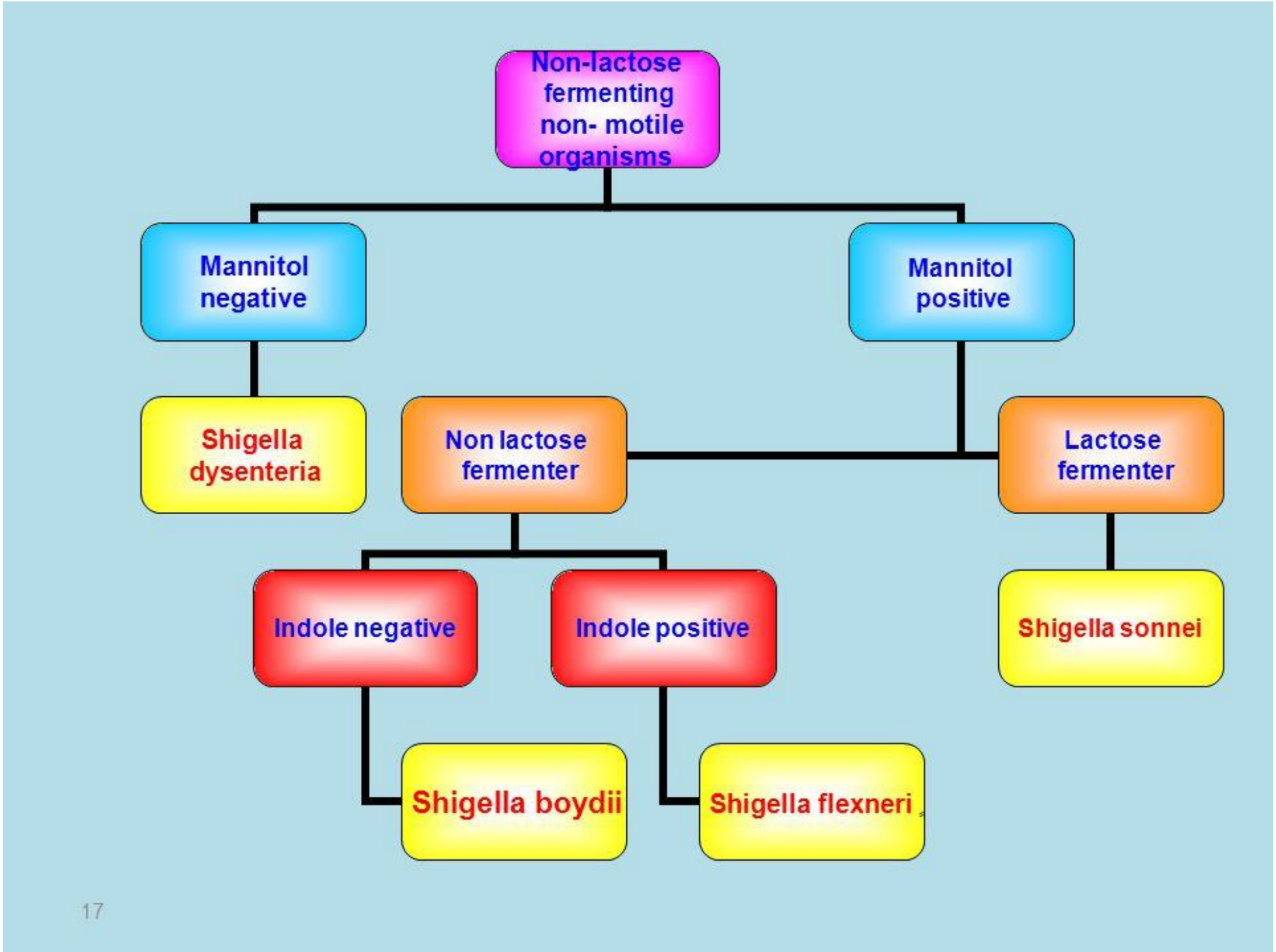
are oxidase negative

CLASSIFICATION
on Basis of Mannitol Fermentation:

- 1. Non-mannitol-fermenters
- *Shigella dysenteria*
- 2. Mannitol-fermenters
- *Shigella flexneri*
- *Shigella boydii*
- *Shigella sonnei*

MORPHOLOGY AND STAINING:

- Short rods
- - Non-encapsulated
- - Non-motile
- - Non-spore former
- - Gram-negative


Non-lactose fermenting non- motile organisms
Mannitol negative
Mannitol positive
Shigella dysenteria
Non lactose fermenter
Lactose fermenter
Indole negative
Indole positive
Shigella sonnei
Shigella boydii
Shigella flexneri

CULTURAL CHARACTERISTICS

- All members of Shigella are aerobic and facultative anaerobes.
- Grow readily in culture media at pH 6.4 to 7.8 at 10 oC - 40 oC, with optimum of 37 oC.
- After 24 hours incubation, Shigella colonies reaches a diameter of about 2 mm.
- The colonies are circular, convex, colorless, but moderately translucent with smooth surface, and entire edges.

HABITAT AND TRANSMISSION

- Shigella species are found only in the human intestinal tract.
- Carriers of pathogenic strains can excrete the organism up to two weeks after infection and occasionally for longer periods.
- Shigella are killed by drying. Shigella are transmitted by the fecal-oral rout.
- The highest incidence of Shigellosis occur in areas of poor sanitation and where water supplies are polluted.

Factors Contributing Spread

- Spread is always from **a human resource** and generally involves one of the five *f*'s:
  - **food,**
  - **fingers,**
  - **feces,**
  - **flies or**
  - **fomites.**
- This is in contrast to salmonellae, which are often spread to humans from infected animals.

PATHOGENESIS

- SOURCE : MAN: CASE OR CARRIER
- MODE OF SPREAD: CONTAMINATED FINGERS, FOOD, FLIES, FOMITES
- PERSON TO PERSON TRANSMISSION
- INFECTIVE DOSE: 10-100 VIABLE BACILLI
- HIGHEST CONCENTRATION IN STOOL DURING EARLY/ACUTE INFECTION 10^3 TO 10^9 VIABLE BACILLI PER GRAM OF STOOL
- POST CONVALESCENT SHEDDING : LOW COUNTS 10^2 TO 10^3

Transmission

- Faecal-oral transmission is the main path of Shigella infection. Other modes of transmission include ingestion of contaminated food or water, contact with infected objects, or sexual contact. Outbreaks of Shigella infection are common in places where sanitation is poor.

Pathogenesis and Virulence Factors (cont.)

Invasiveness in *Shigella*-Associated Dysentery

- Penetrate through mucosal surface of colon (colonic mucosa) and invade and multiply in the colonic epithelium but do not typically invade beyond the epithelium into the lamina propria (thin layer of fibrous connective tissue immediately beneath the surface epithelium of mucous membranes)
- Preferentially attach to and invade into M cells in Peyer's patches (lymphoid tissue, i.e., lymphatic system) of small intestine

Invasiveness in *Shigella*-Associated Dysentery (cont.)

- M cells typically transport foreign antigens from the intestine to underlying macrophages, but *Shigella* can lyse the phagocytic vacuole (phagosome) and replicate in the cytoplasm
  - Note: This contrasts with *Salmonella* which multiplies in the phagocytic vacuole
- Actin filaments propel the bacteria through the cytoplasm and into adjacent epithelial cells with cell-to-cell passage, thereby effectively avoiding antibody-mediated humoral immunity (similar to *Listeria monocytogenes*)

Pathogenesis & Immunity

- **Exotoxin (Shiga toxin)** is neurotoxic, cytotoxic, and enterotoxic, encoded by chromosomal genes,
- **Enterotoxic effect**: Shiga toxin adheres to small intestine receptors
- Blocks absorption (uptake) of electrolytes, glucose, and amino acids from the intestinal lumen

Pathogenesis & Immunity

- Cytotoxic effect: B subunit of Shiga toxin binds host cell glycolipid in large intestine,
- Inactivate the 60S ribosomal subunit,
- Inhibit protein synthesis, causing cell death, microvasculature damage to the intestine, and hemorrhage (blood and fecal leukocytes in stool)
- Neurotoxic effect: Fever, abdominal cramping are considered signs of neurotoxicity

Pathogenesis and Virulence Factors (cont.)

Shiga Toxin Effects in Shigellosis

Enterotoxic Effect:

- Adheres to small intestine receptors
- Blocks absorption (uptake) of electrolytes, glucose, and amino acids from the intestinal lumen
  - Note: This contrasts with the effects of cholera toxin (*Vibrio cholerae*) and labile toxin (LT) of enterotoxigenic *E. coli* (ETEC) which act by blocking absorption of Na^+, but also cause hypersecretion of water and ions of Cl^-, K^+ (low potassium = hypokalemia), and HCO_3^- (loss of bicarbonate buffering capacity leads to metabolic acidosis) out of the intestine and into the lumen

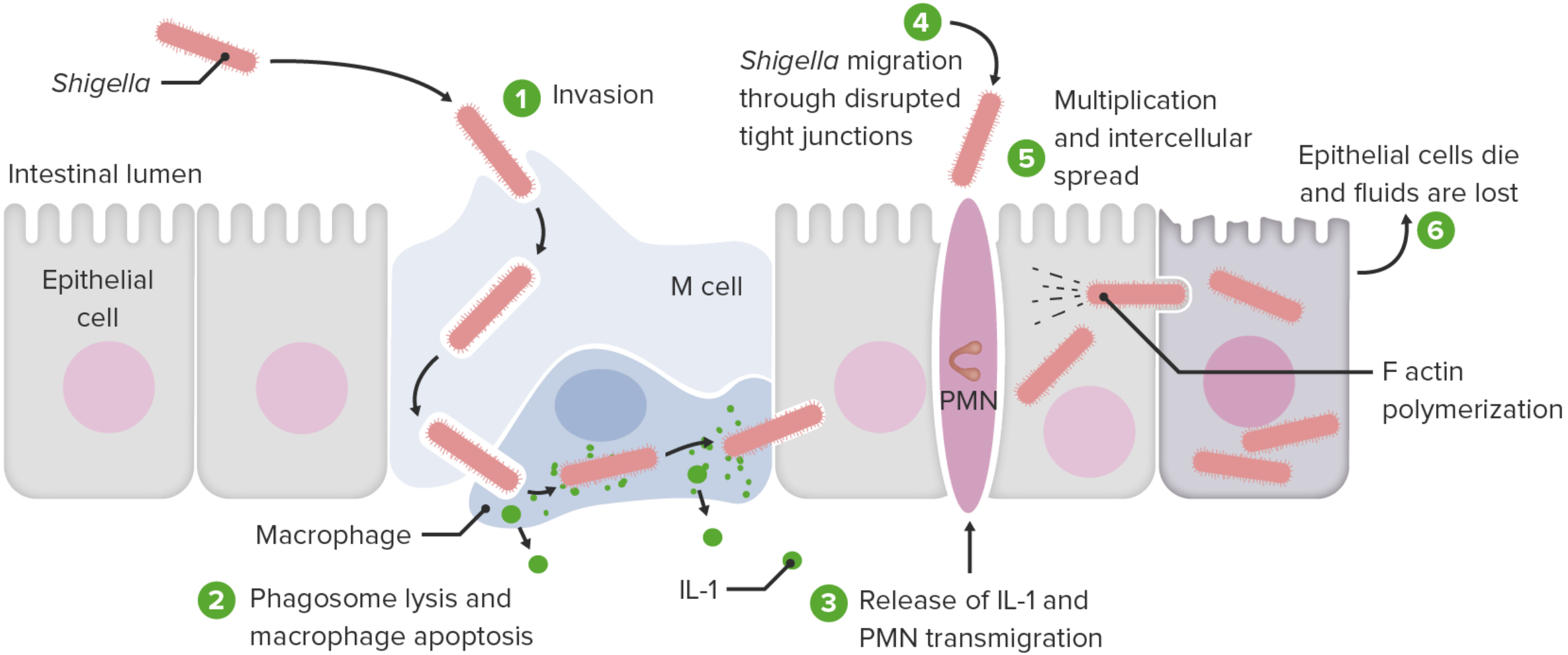
Pathogenesis and Virulence Factors (cont.)

Shiga Toxin Effects in Shigellosis (cont.)

Cytotoxic Effect:

- B subunit of Shiga toxin binds host cell glycolipid
- A domain is internalized via receptor-mediated endocytosis (coated pits)
- Causes irreversible inactivation of the 60S ribosomal subunit, thereby causing:
  - Inhibition of protein synthesis
  - Cell death
  - Microvasculature damage to the intestine
  - Hemorrhage (blood & fecal leukocytes in stool)

Neurotoxic Effect: Fever, abdominal cramping are considered signs of neurotoxicity


Shigella
1 Invasion
Intestinal lumen
Epithelial cell
M cell
Macrophage
2 Phagosome lysis and macrophage apoptosis
IL-1
3 Release of IL-1 and PMN transmigration
4 Shigella migration through disrupted tight junctions
5 Multiplication and intercellular spread
PMN
Epithelial cells die and fluids are lost
6
F actin polymerization

Clinical Syndromes (Shigellosis)

- Ranges from asymptomatic infection to severe bacillary dysentery
- **Two-stage disease:** watery diarrhea changing to dysentery with frequent small stools with blood and mucus, tenesmus, cramps, fever

Early stage:

- Watery diarrhea attributed to the enterotoxic activity of Shiga toxin
- Fever attributed to neurotoxic activity of toxin

Epidemiology

- Shigellosis is a major cause of diarrheal disease (developing nations)
- Major cause of bacillary dysentery (severe second stage form of shigellosis)
- Leading cause of infant diarrhea and mortality (death) in developing countries

LABORATORY DIAGNOSIS

The only satisfactory method of laboratory diagnosis is to cultivate the bacilli from the patient.

In the early stages of acute shigellosis, isolation of the causative organism from the feces is usually accomplished without difficulties by using the same special media and methods employed for salmonella

Laboratory Findings

- **Blood picture:**

  WBC count increase,
  (10~20×10⁹/L)
  neutrophils increase

- **Stool examination:**
  - gross examination: stool mixed with mucus, blood & pus.

Laboratory Findings

- direct microscopic examination:
  WBC, RBC, pus cells
- bacteria culture:
- PCR:DNA

Sigmoidoscopy:

chronic patients

shallow ulcer

scar

polyp

Microbiological diagnosis:

- *Pathological material:*

stool

- *Bacteriological (cultural) method:*

✓examination materials (stool) are inoculated into differential-diagnostic nutrient media containing lactose (Endo, Levin, Ploskirev, MacConkey media).

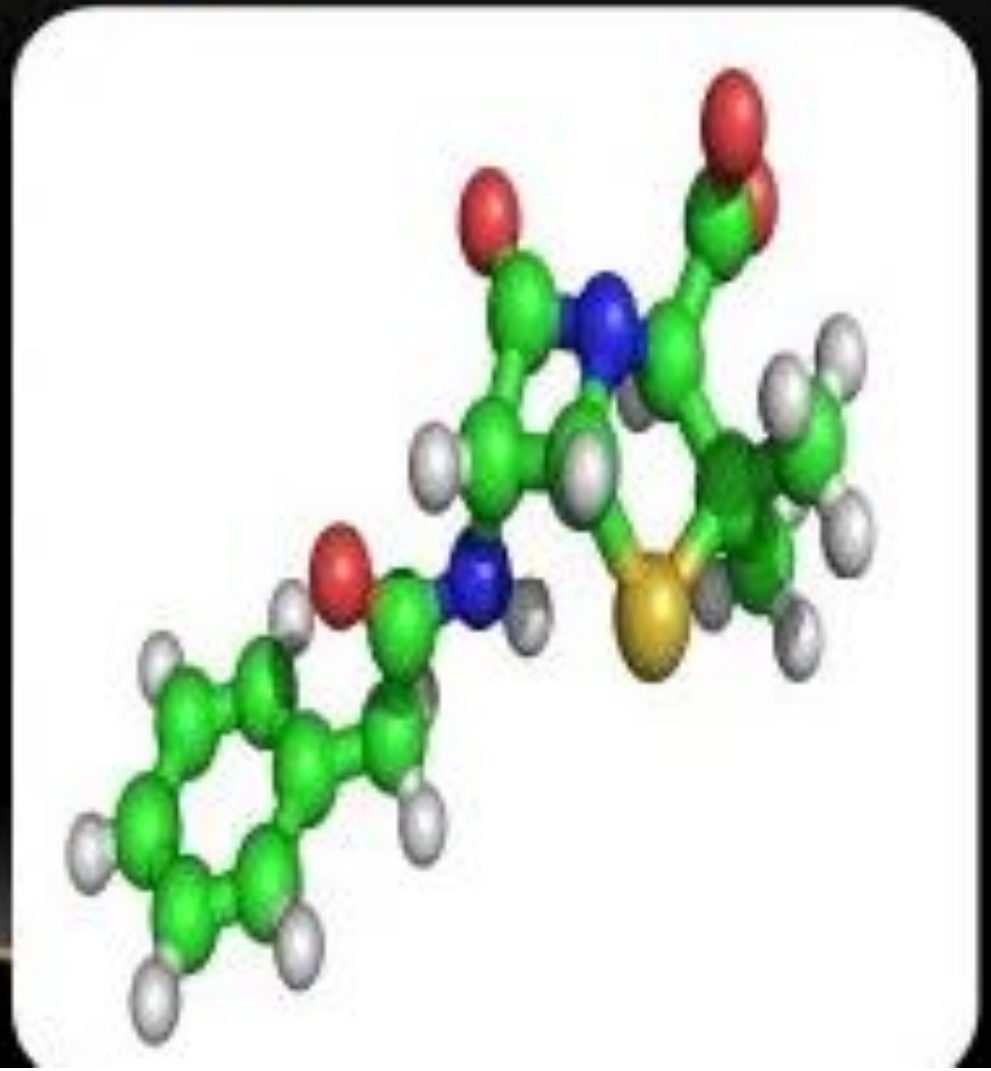
✓It is incubated at 37ºC for 18-24 hours.

✓developed lactose negative colonies are identified based on their morphological, biochemical and antigenic properties.

✓sensitivity to antibiotics is determined.

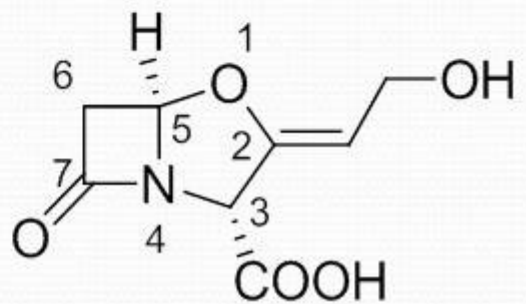
WHAT ARE EXTENDED-SPECTRUM B-LACTAMASES?

- ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and Cefotetan) or carbapenems (e.g., meropenem or imipenem).



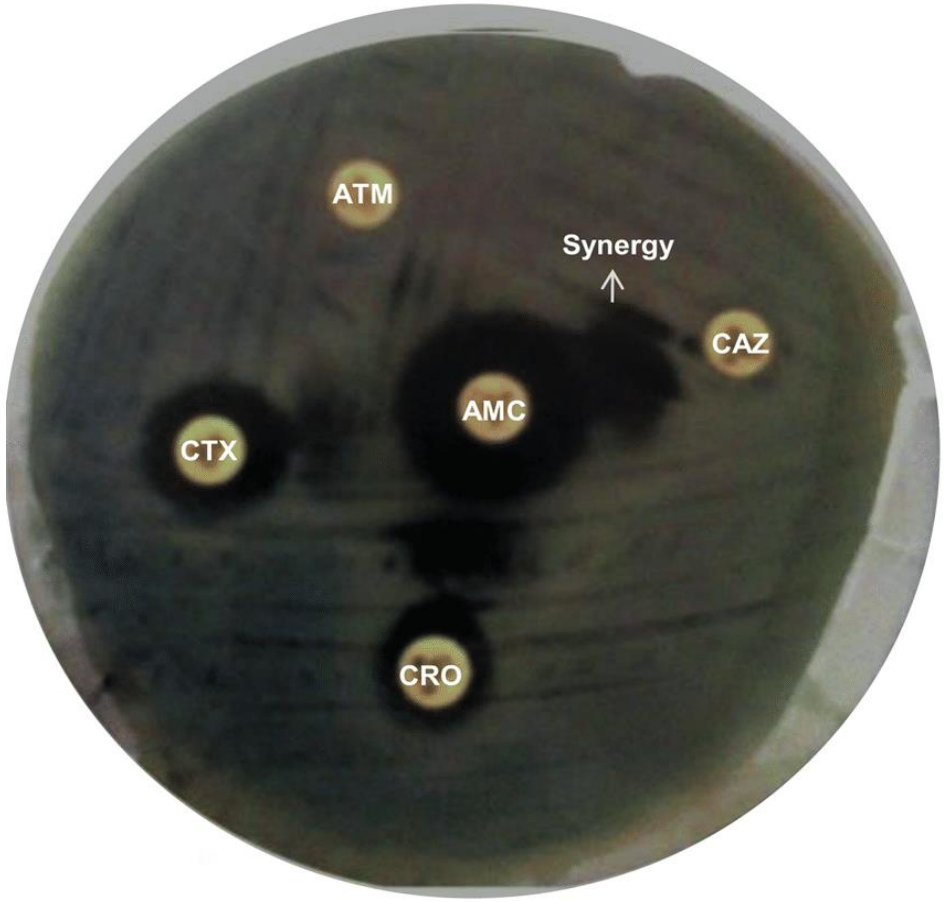
β-lactamase inhibitors

- Almost all have weak antibacterial activity.
- Important in combination with penicillins sensitive to β-lactamase degradation.
- Clavulanic acid is the first one of this class.
  - Natural product from streptomyces.
  - Has a powerful and irreversible inhibition of β-lactamase enzymes because it will covalently bind to two positions in the active site.
  - Normally used in combination with amoxicillin and other β-lactamase sensitive penicillins



Beta-Lactamase Inhibitors

- Clavulanic acid, Tazobactam, Sulbactam
- **Drug Class:** inhibitors of beta-lactamase
- **Trade Names:**
  - Augmentin ® (Amoxicillin + Clavulanate)
  - Zosyn ® (Piperacillin + Tazobactam)
  - Timentin ® (Ticarcillin + Clavulanate)
- **Mechanism of Action:**
  - these three substances resemble β-lactam molecules & are **potent inhibitors of "most" plasmid-mediated beta-lactamases**.
  - **Sulbactam has intrinsic activity against Acinetobacter & may be used against MDR strains.**
- **Indications:**
  - used in fixed combination with specific penicillins: ampicillin, amoxicillin or ticarcillin
  - **penicillin-β-lactamase inhibitor combinations are used for empirical therapy against a wide range of potential pathogens including treatment of aerobic & anaerobic infections (e.g. intra-abdominal infections)**.
  - The β-lactam inhibitor merely extends the activity of the combined penicillin



Phenotypic detection of ESBL producing E. coli. The AMC (amoxicillin/clavulanic acid) disc was placed at the center of the agar plate, while the discs of ATM (aztreonam), CAZ (ceftazidime), CRO (ceftriaxone), and CTX (cefotaxime) were placed in close proximity. The resistance of the E. coli isolate to all cephalosporin and aztreonam, and the synergy between AMC and CAZ, phenotypically confirmed the ESBL production.

Taxonomy

Domain: Bacteria
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Vibrionales
Family: Vibrionaceae
Genus: *Vibrio*

- *Vibrio cholerae*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Vibrio hollisae*
- *Vibrio mimicus*
- *Vibrio metschnikovii*
- *Vibrio alginolyticus*

TAXONOMY:

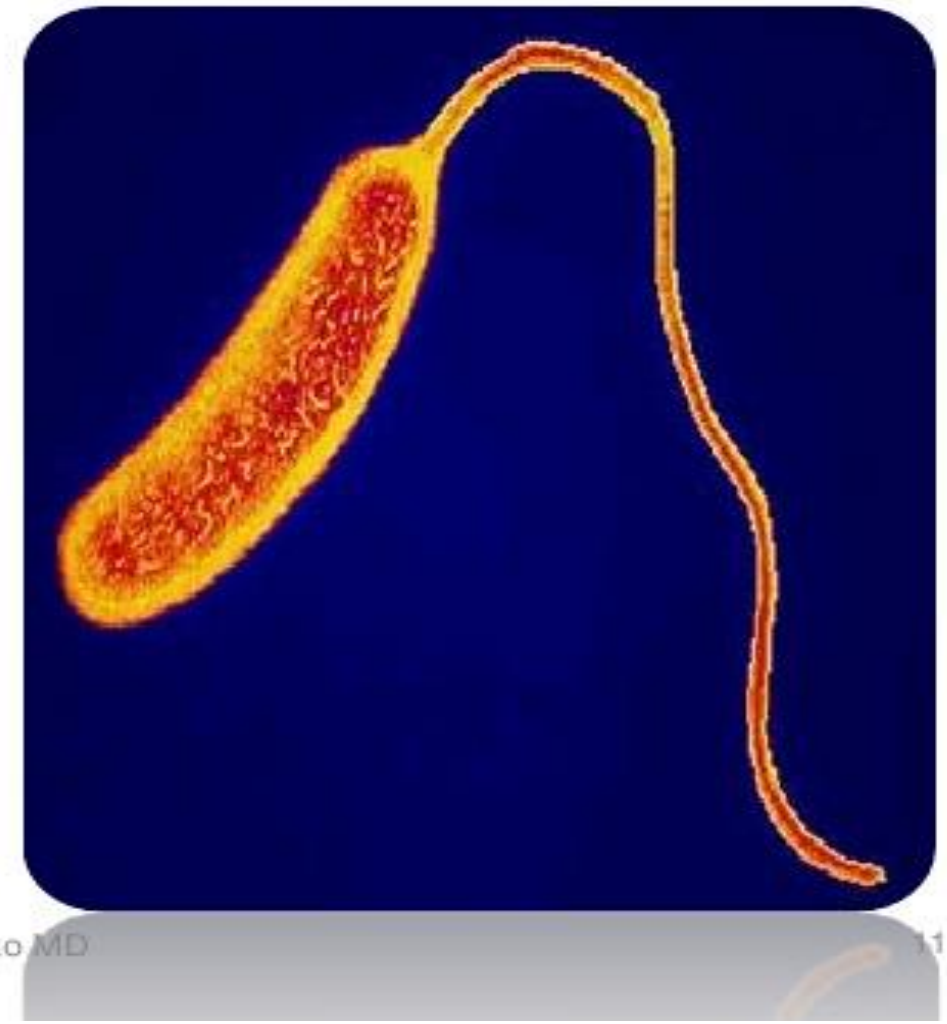
- class : Gamma Proteobacteria
- Order: Vibrionales
- Family: Vibrionaceae
- Genus: Vibrio
- Species: v.cholerae, v.parahaemolyticus, v. vulnificus, v. alginolyticus

Vibrio cholerae

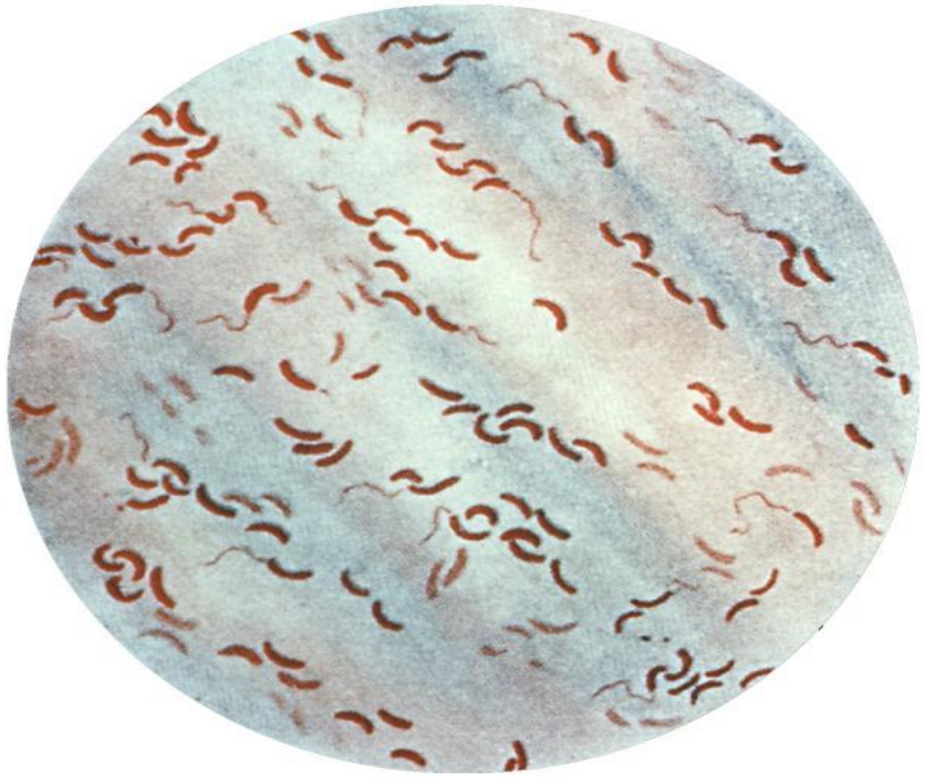
Smooth colony morphology

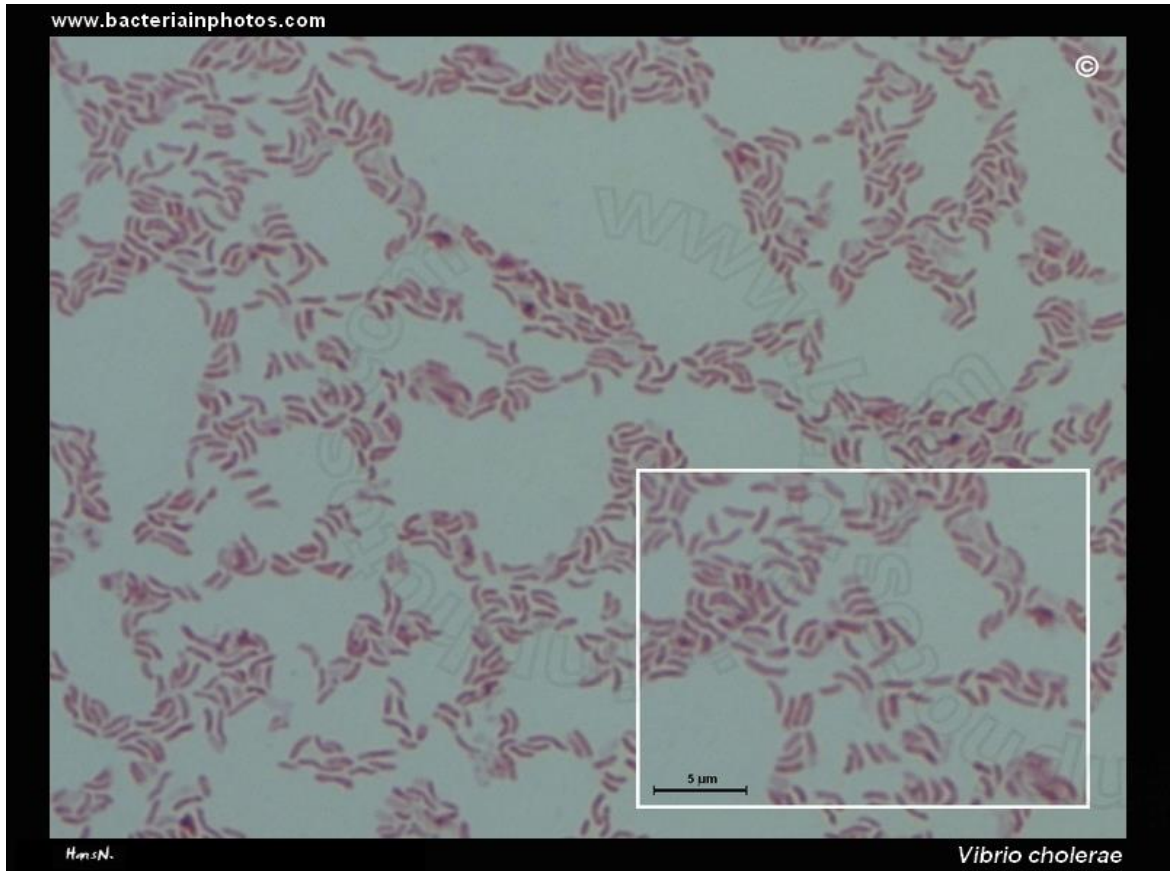
Morphology and Identification

- V.cholrae is a comma shaped curved rod 2 – 4 µm long'
- It is actively motile by means of polar flagellum.
- On prolonged cultivation, vibrio's may become straight rods that resemble the gram-negative enteric bacteria.



Vibrio cholera





MORPHOLOGY:

- Gram negative, actively motile, short, rigid curved bacilli
- Resembling letter "V"
- about 34 genus
- most common in water
- 1.5µ X 0.2 -0.4 µ in size
- polar flagellum , strongly aerobic
- Smear – fish in stream appearance

Cultural characteristics

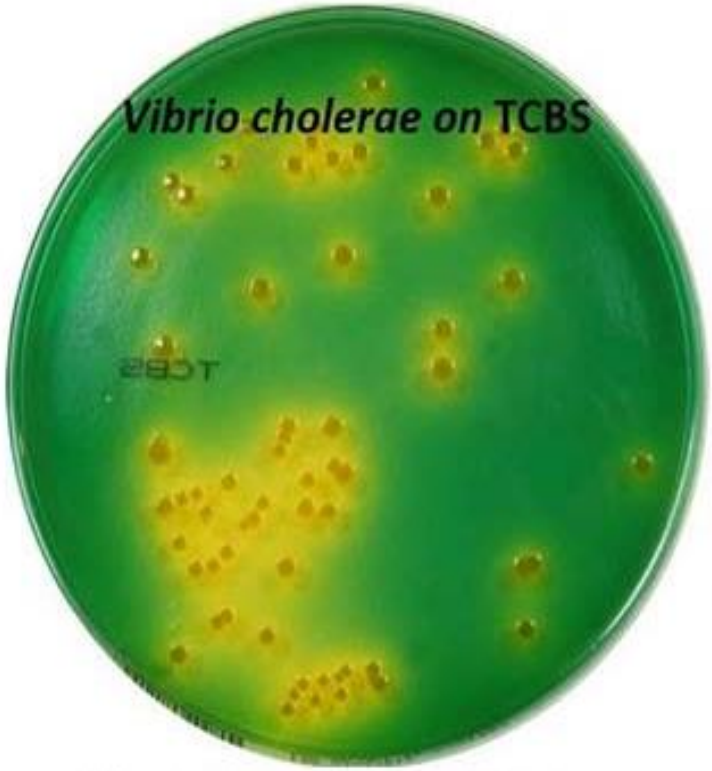
- The cholera vibrions is strongly aerobic
- Growth is better in an alkaline medium the range of pH being 6.4-9.6 (optimum 8.2)
- Special media:
- Alkaline peptone water at pH 8.6;

2. Culture & Growth

- *V cholerae* produces convex, smooth, round colonies that are opaque and granular in transmitted light.
- *V cholerae* and most other vibrios grow well at 37°C on many kinds of media.
- *V cholerae* grows well on **thiosulfate-citrate-bile-sucrose (TCBS)** agar, a media selective for vibrios, on which it produces yellow colonies (sucrose fermented) that are readily visible against the dark-green background of the agar

Vibrio cholerae

(TCBS – thiosulfate citrate bile sucrose agar)



V.cholerae

V.parahaemolyticus

ANTIGENIC STRUCTURE

- Heat-labile flagellar H antigen.
- O-lipopolysaccharides has 140 serogroups.
- Serogroup O1 and O139-classic cholera.
- Others cause cholera like diseases.
- Serotypes (inaba,ogawa,hikojima) and biotypes(classical,el tor)
- El tor- hemolysin.
- Serogroup O139- polysaccharide capsule.

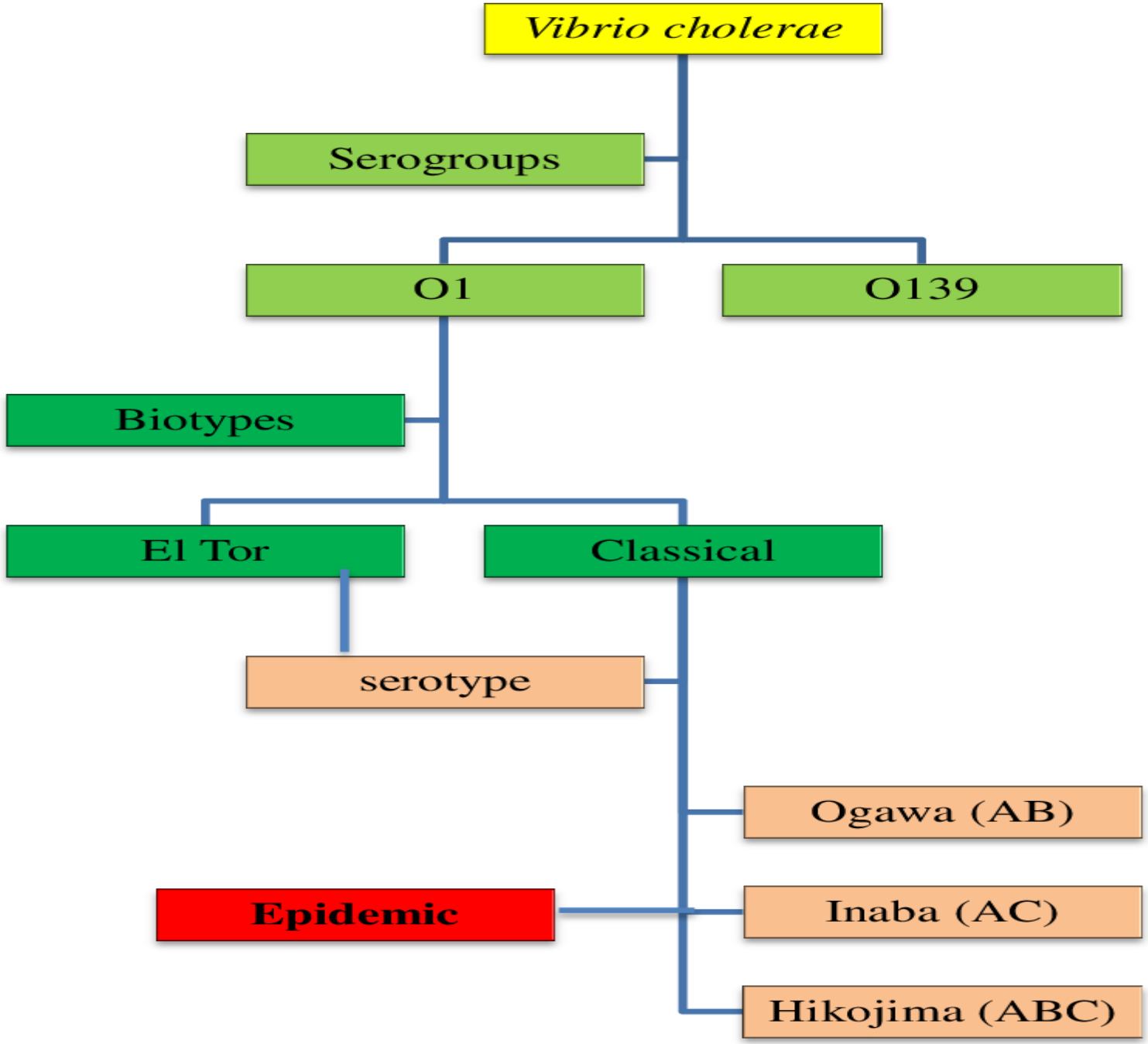

Vibrio cholerae
Serogroups
O1
O139
Biotypes
El Tor
Classical
serotype
Ogawa (AB)
Epidemic
Inaba (AC)
Hikojima (ABC)

TABLE 35-2 Differential characteristics of *Vibrio cholerae* biotypes

Properties	*Vibrio cholerae* biotype	
	Classical	Eltor
Hemolysis of sheep RBCs	–	+
Agglutination of chick erythrocytes	–	+
Voges–Proskauer test	–	+
Polymixin B sensitivity	+	–
Susceptibility to		
Mukerjee Group IV Phage	+	–
Eltor phage 5	–	+
Vibriostatic (O/129) agent	+	–

TABLE 35-4 Virulence factors of *Vibrio cholerae*

Virulence factors	Biological functions
Cholera toxin	The toxin inhibits absorption of sodium and chloride in the intestine; causes hypersecretion of large volumes of water and electrolytes. Activation of adenylate cyclase and overproduction of cAMP.
Toxin coregulated pilus	Helps in adherence of *Vibrio cholerae* to mucosal cells of the intestine
Accessory colonization	Helps in adhesion of bacteria to the intestinal mucosa
Hemagglutination-protease (mucinase)	Induces intestinal inflammation and also helps in releasing free vibrios from the bound mucosa to the intestinal lumen
Neuraminidase	Increases toxin receptors for *Vibrio cholerae*
Siderophores	Causes sequestration of iron

Modes of Transmission

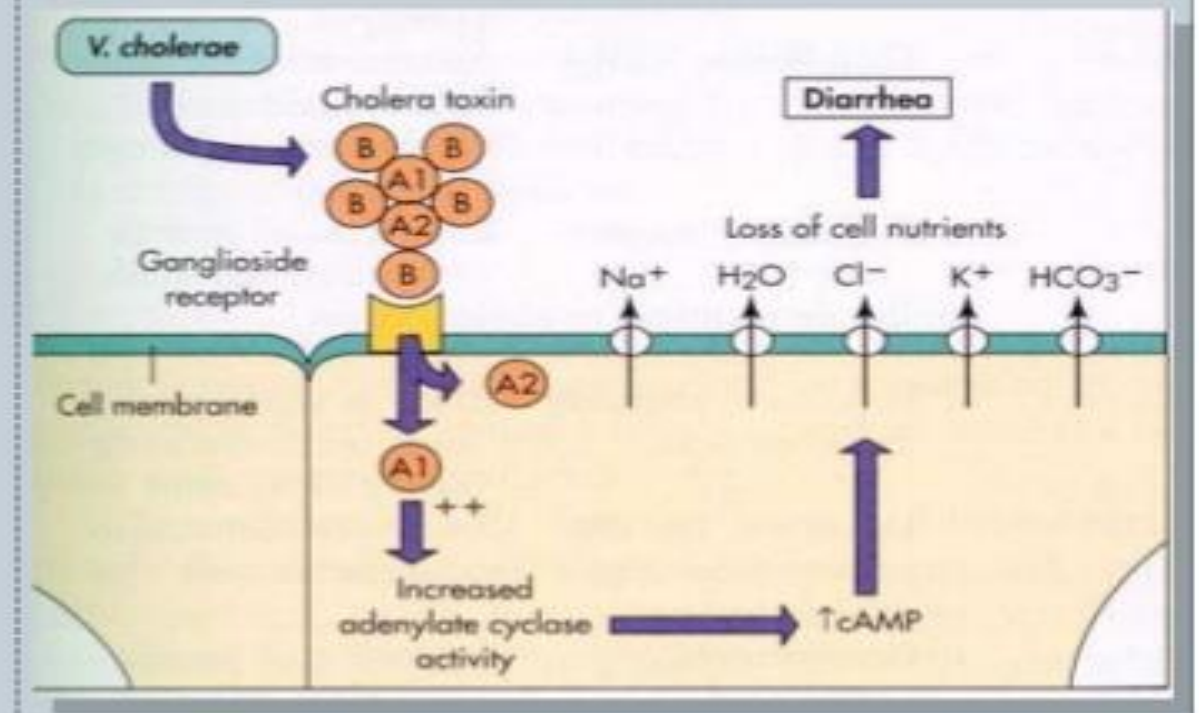
- Water (infectious dose = 10^9)
- Food (infectious dose = 10^3)
- Person-to-person

The 19th-century illustration depicting the spirit of death at a pump was taken from *http://*news.nationalgeographic.com/news/2004/06/0614_040614_tvcholera.html

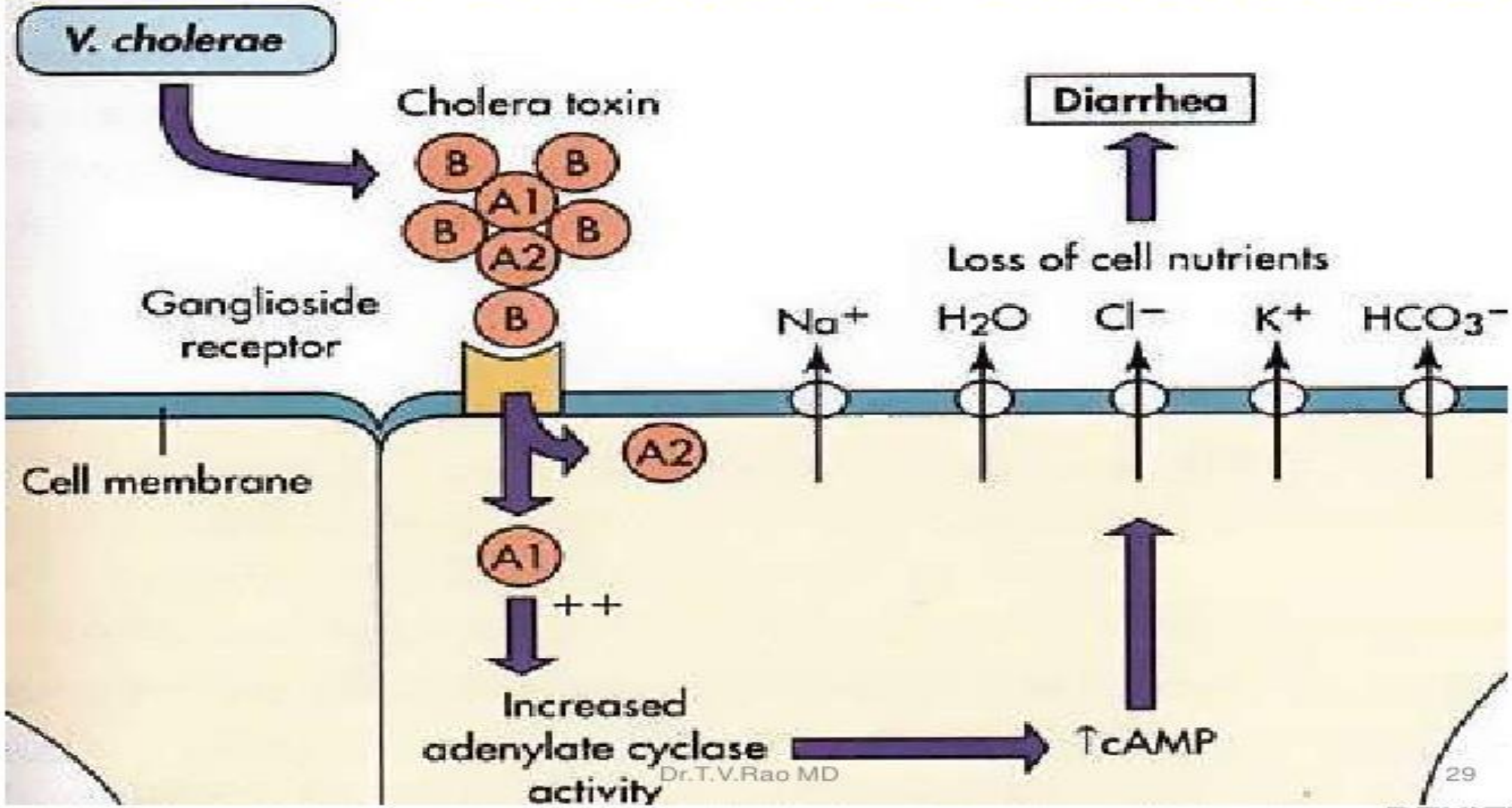
Pathogenesis

Vibrio Cholerae

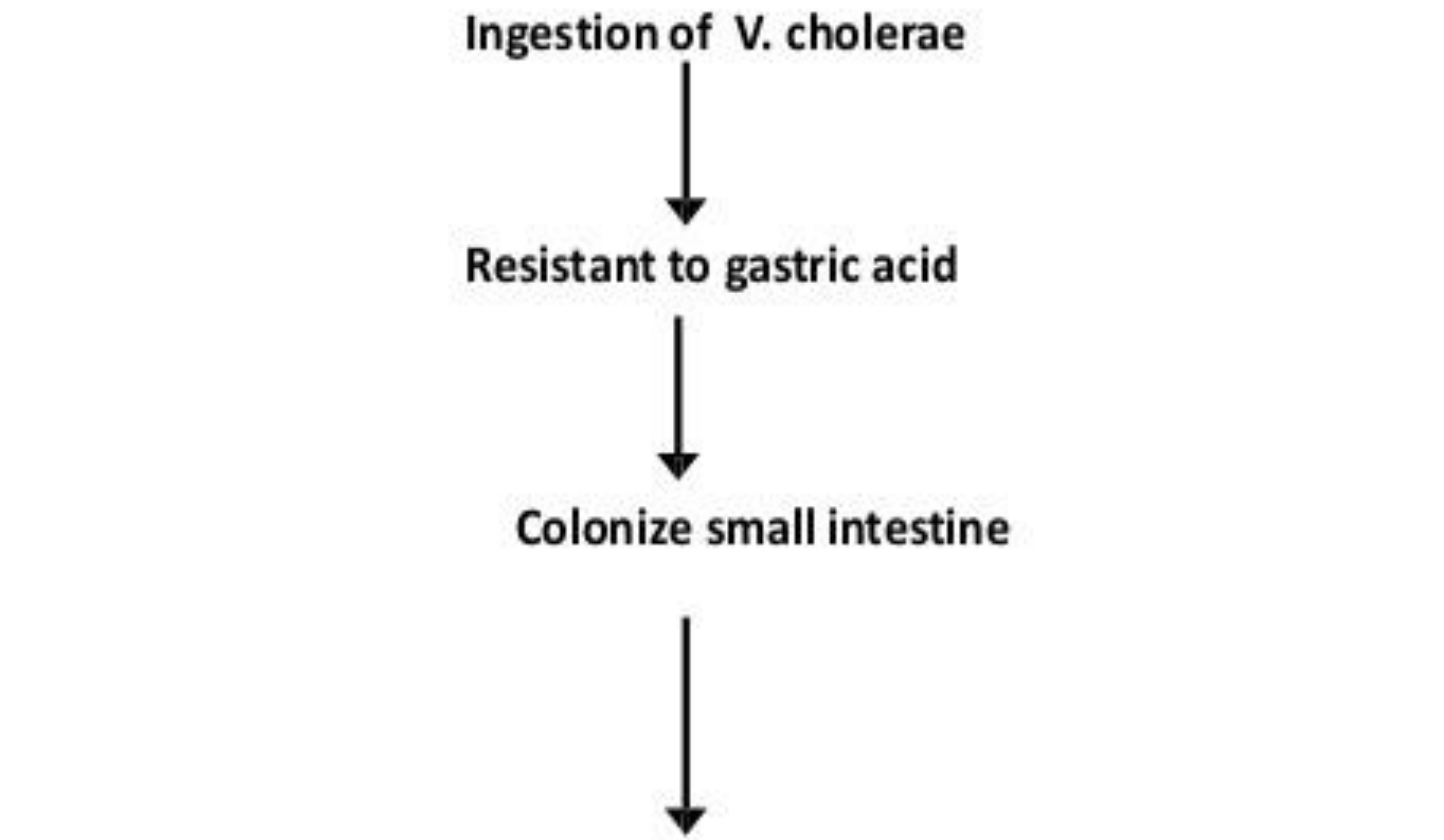
- Vibrio Cholerae enterotoxin activates the stimulatory Gs protein via ADP-ribosylation. This stimulates secretion of chloride ions and water from enterocytes into the small intestines, and causing watery diarrhea.

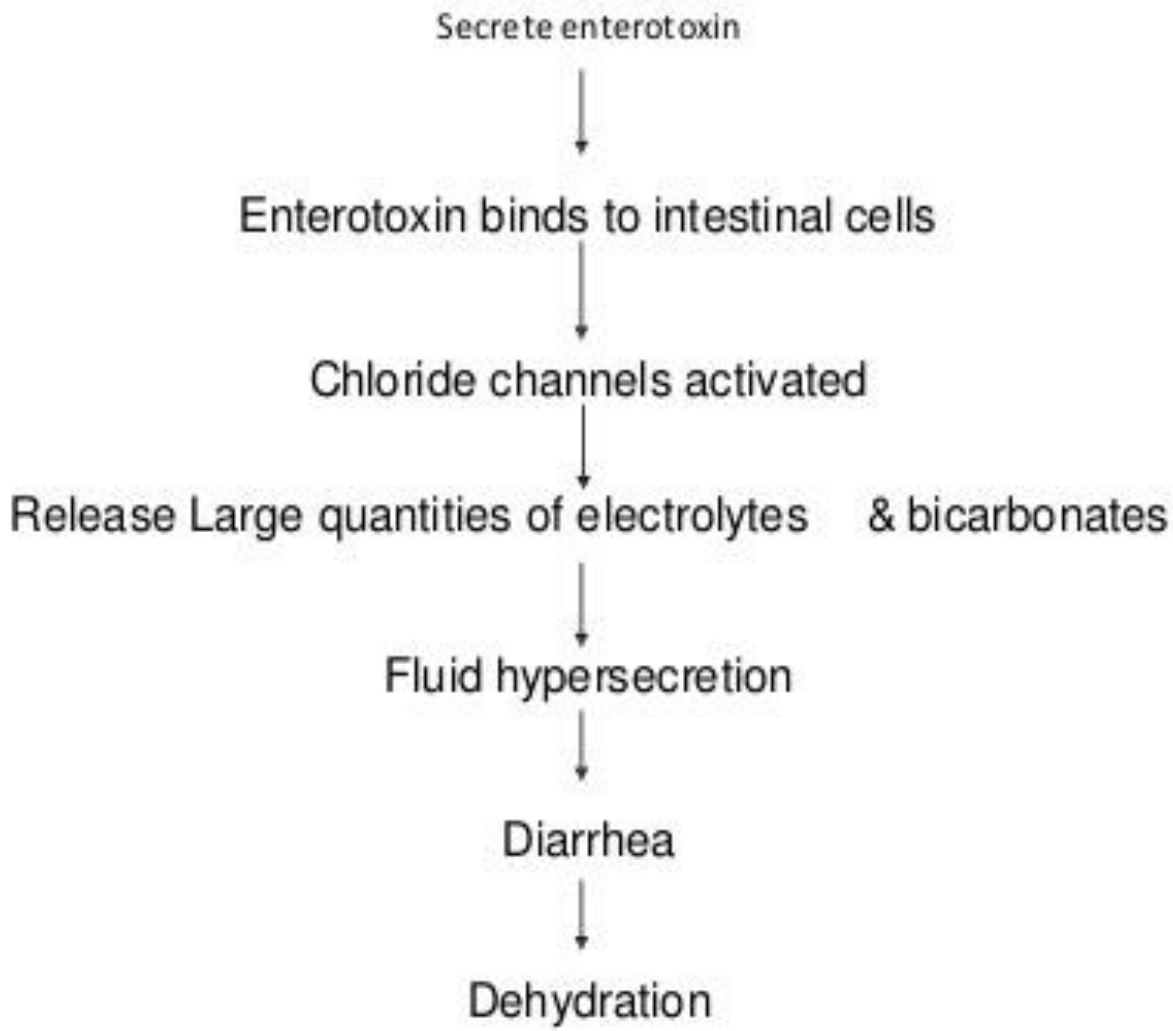


Mechanism of Action of Cholera Toxin



virulence &pathogenicity




Secrete enterotoxin
Enterotoxin binds to intestinal cells
Chloride channels activated
Release Large quantities of electrolytes & bicarbonates
Fluid hypersecretion
Diarrhea
Dehydration

CHOLERA

- Cholera is an acute diarrheal illness caused by infection of the intestine with the bacteria Vibrio cholerae.

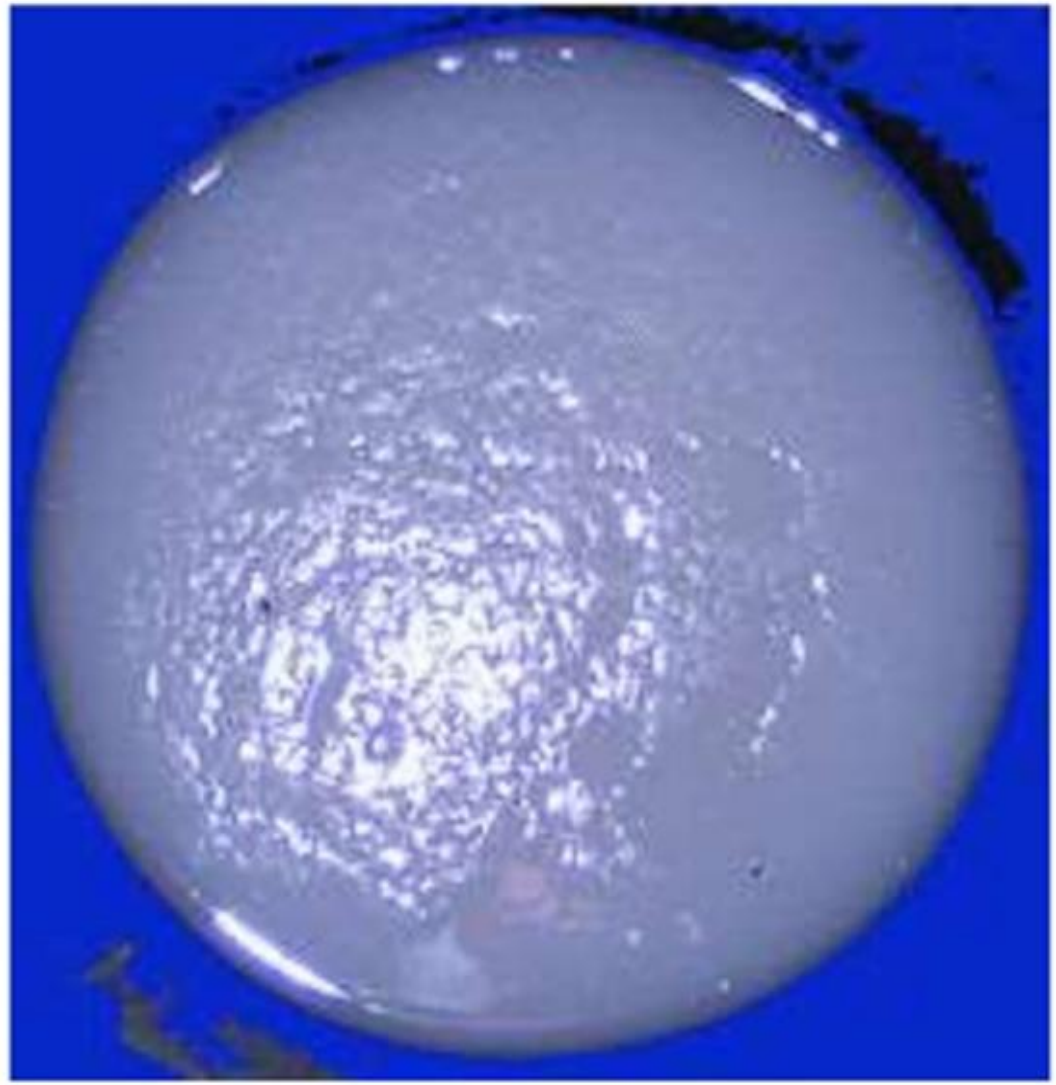
Symptoms

- Usually mild, or no symptoms at all
    - 75% asymptomatic
    - 20% mild disease
    - 2-5% severe
- Vomiting
- Cramps

– **profuse, painless diarrhea and vomiting of clear fluid.**

"rice water" (1L/hour) >20 mL/kg during a 4-hour observation period

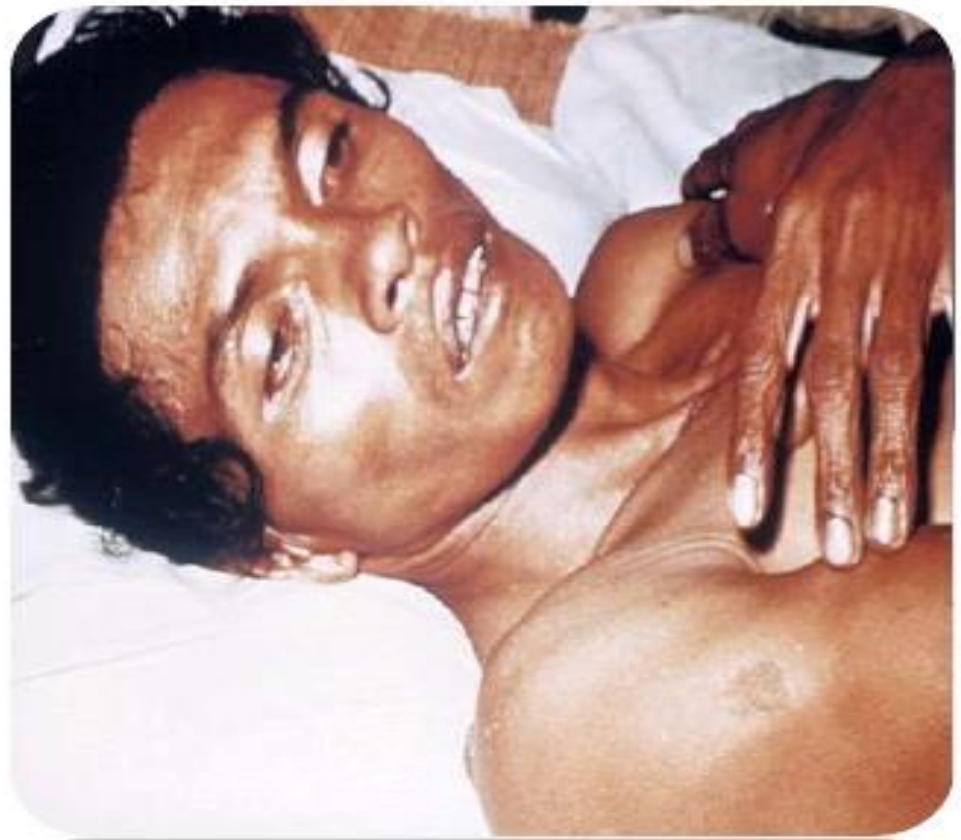
- Without treatment, death in 18 hours-several days



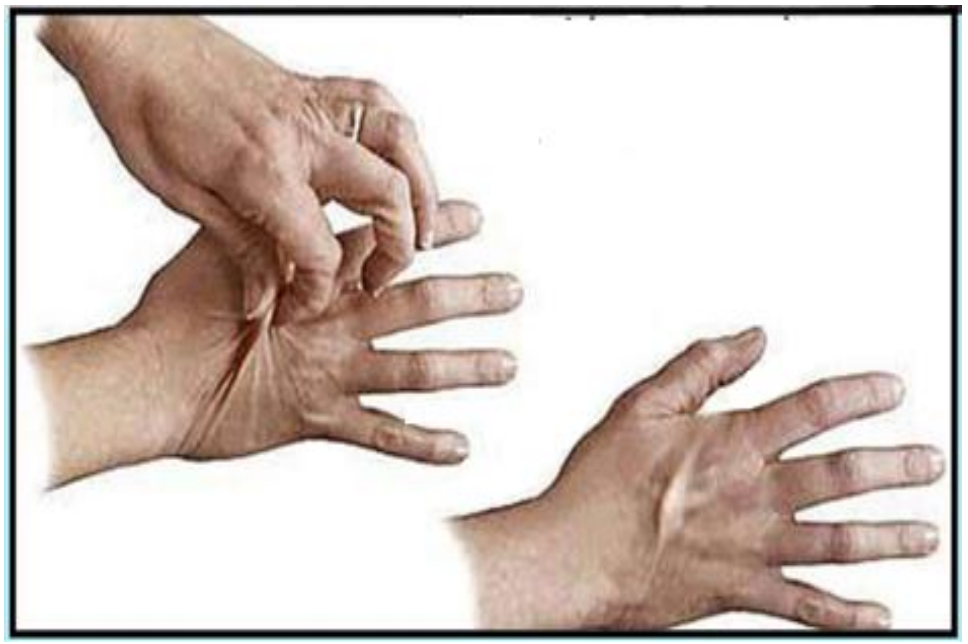
- **Typical "rice water" diarrhea**

Clinical manifestations

- Diarrhea occurs as much as 20 – 30 Liters/Day fluids are lost.
- Results in dehydration
- Shock
- Acidosis
- Can lead to death.
- About 60% of infections are caused with classic V.cholrae and are asymptomatic, about 75% of infections are caused by El Tor biotype

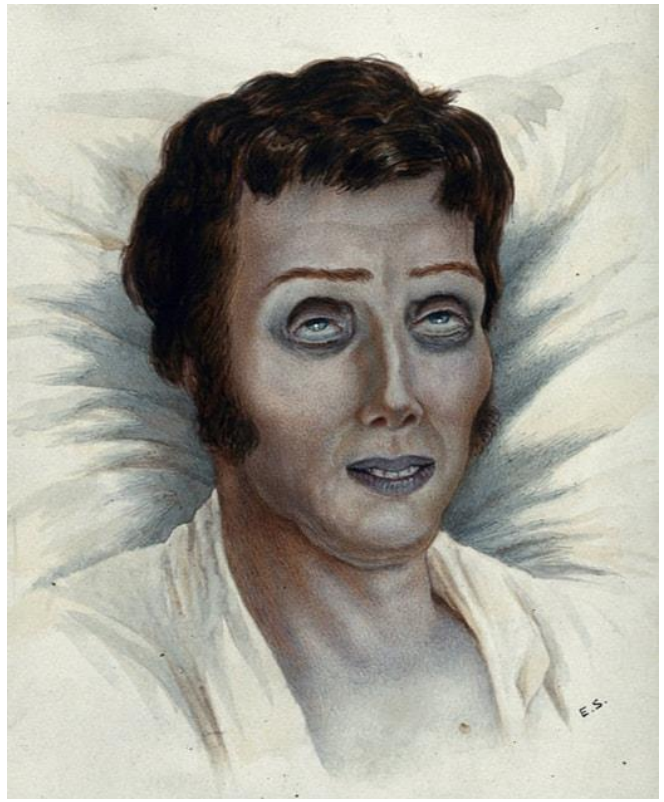


CHOLERA



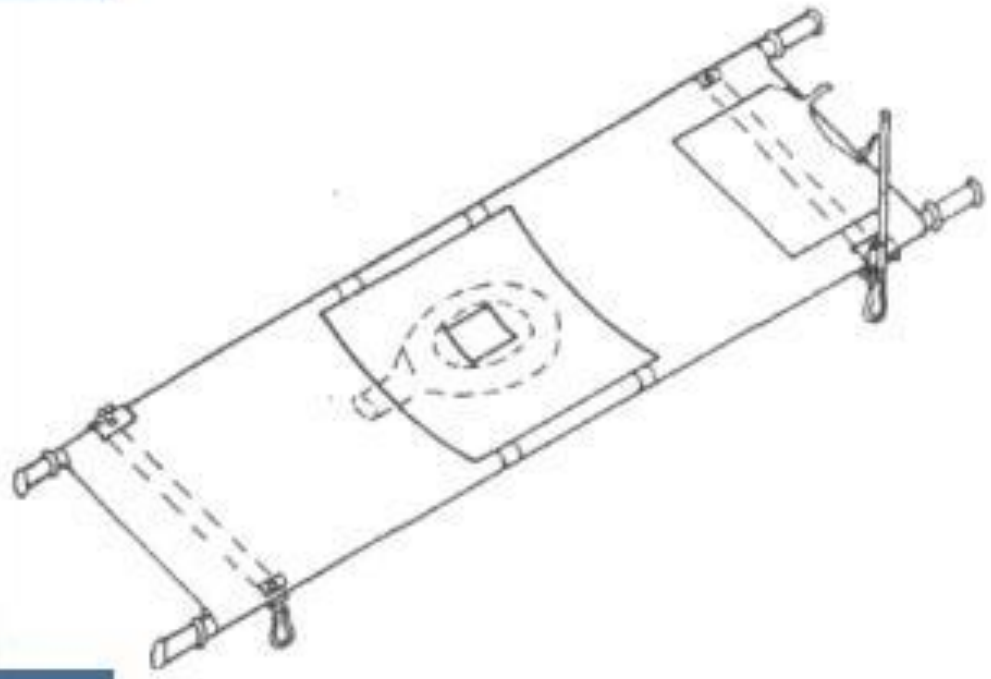
Loss of skin turgor

The hand that washes clothes



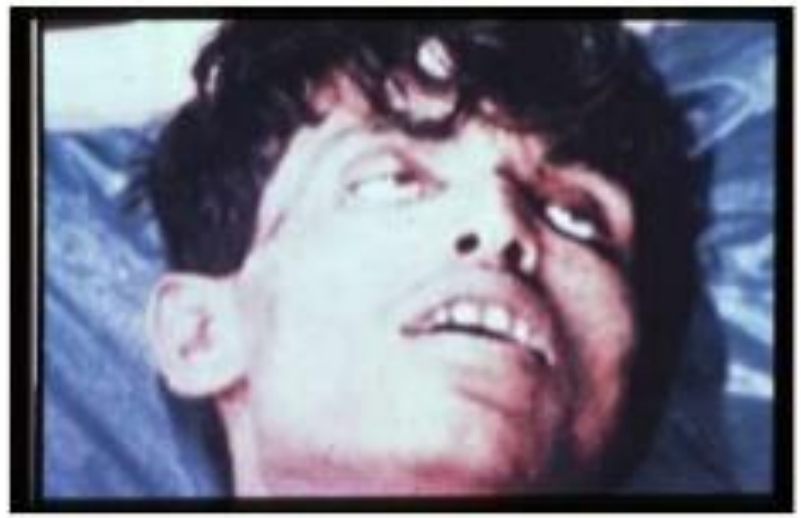
"setting sun" and "dark glasses" symptom

Special beds for patients with cholera



Consequences of Severe Dehydration

1. Intravascular volume depletion
2. Severe metabolic acidosis
3. Hypokalemia →cardiac arrest
4. low blood sugar (hypoglycemia)
    1. Seizures
    2. coma, especially in the young
5. Cardiac and renal failure
6. Sunken eyes, decreased skin turgor
7. Almost no urine production



Diagnosis

- Stool culture: Toxigenic *Vibrio cholerae* O1
- Use Cary Blair Transport media if available
  - Viable for many days at room temperature
- Use TCBS media for culture
- Use *V. cholerae* serogroup O1 antisera
- Confirm presence of cholera toxin
- Cholera Rapid Test Dipsticks

Laboratory diagnosis:

Clinical specimens: vomitus, stool.

Cultural characteristics:

-*Vibrio cholera* species are non-Halophilic.

-They grow on different media **at high pH** (8.5-9.5) as alkaline peptone water.

- **TCBS** is a selective medium.
- On MacConkey's agar : non-lactose fermenting colonies.
- On blood agar:

  Usually beta haemolytic.
- All *Vibrio* species are :

  Facultative anaerobic.

Treatment

REHYDRATION

- Oral
- Intravenous

ANTIMICROBIAL THERAPY

Campilobacter - TAXONOMY

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Proteobacteria

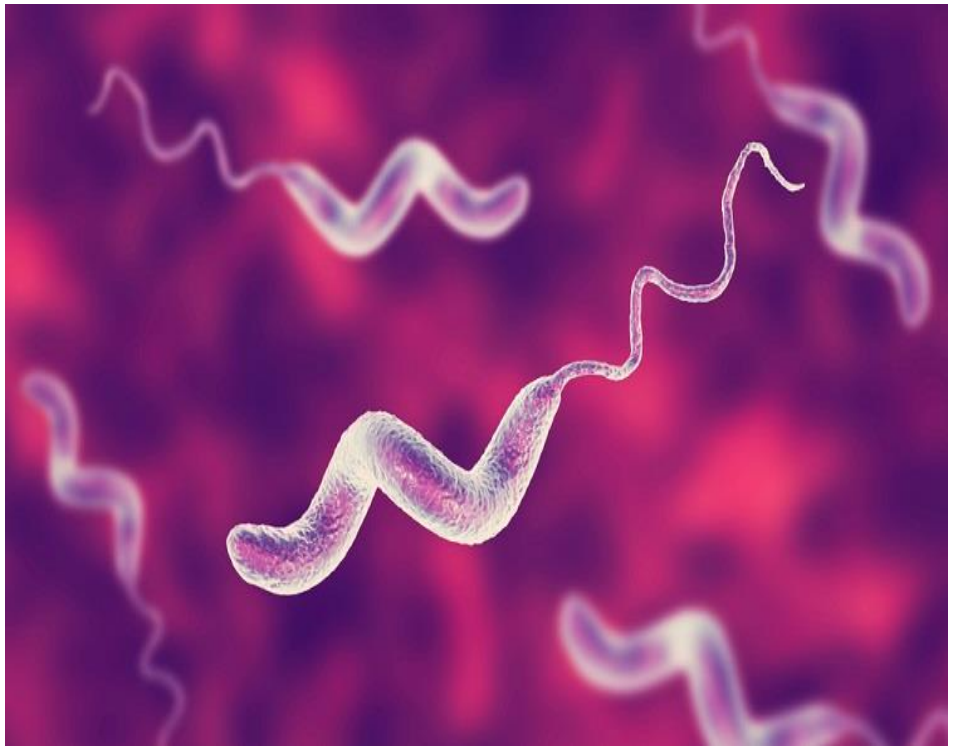
Class: Epsilonproteobacteria

Order: Campylobacterales

Family: *Campylobacteriaceae*

Genera: *Campylobacter*

Species: ***jejuni, coli, fetus***



Campylobacter

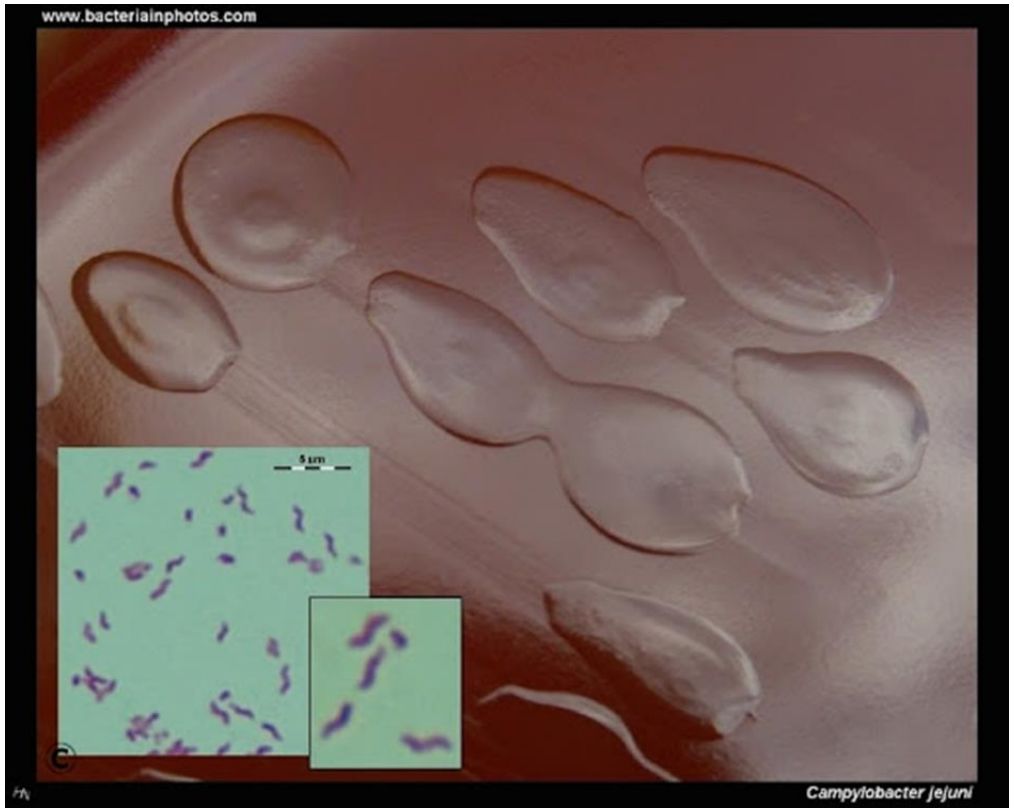
- Among the most widespread cause of infection in the world.
- Cause both diarrheal and systemic diseases
- *Campylobacter jejuni*

Typical Organisms

- Gram-negative rods with comma, S, or “gull-wing” shapes.
- Motive, with a single polar flagellum
- No spore & no capsule

Culture

- An atmosphere with reduced O_2 (5% O_2) with added CO_2 (10% CO_2)
- At 42 ℃ (for selection)
- Several selective media can be used (eg, Skirrow's medium)
- Two types of colonies:
  - ☺ watery and spreading
  - ☺ round and convex



Biochemical tests of Campylobacter

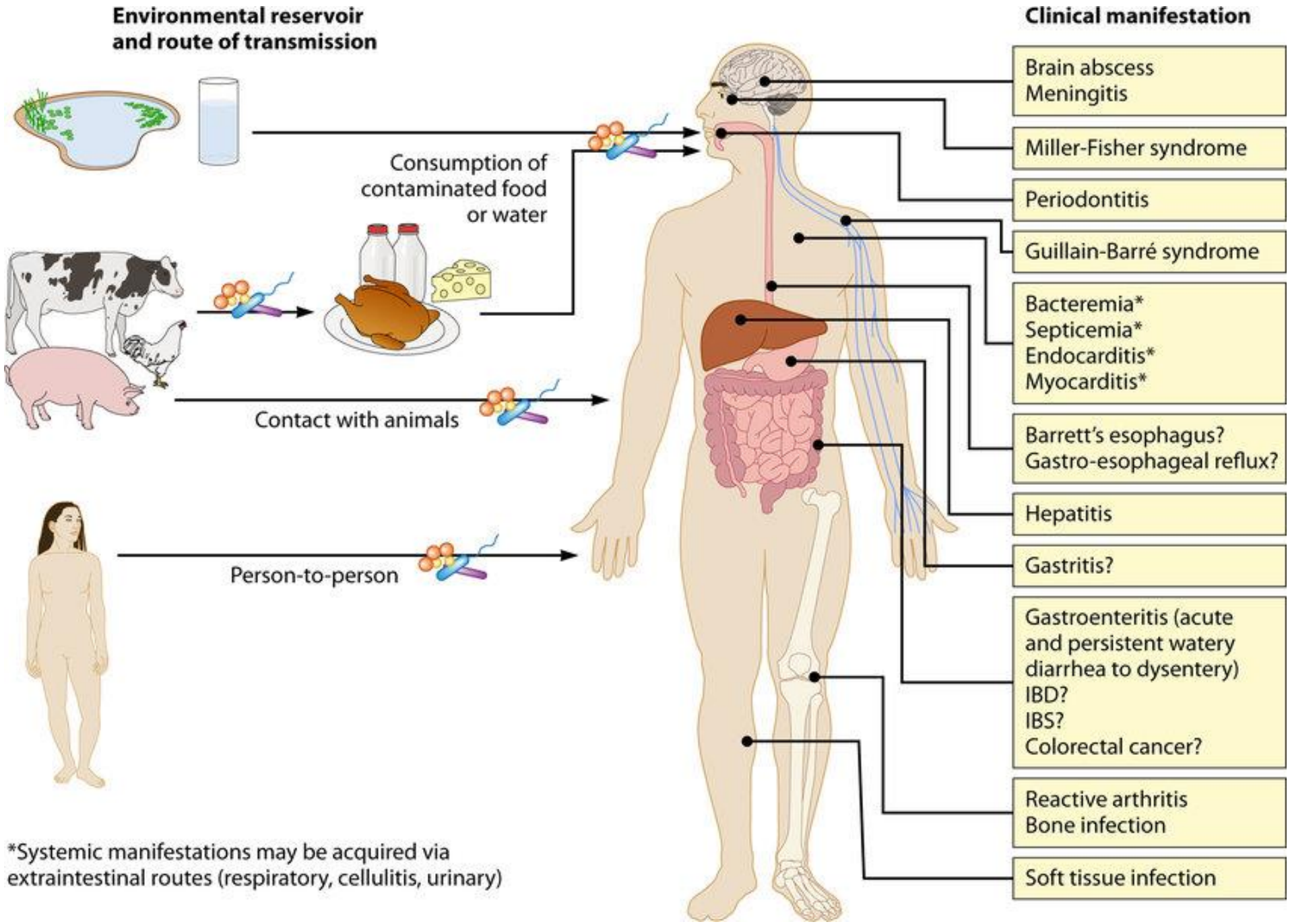
Organism	Biochemical Tests				
	Catalase	**Oxidase**	**KOH**	**Indoxyl acetate**	**Hippurate**
C. jejuni	+	+	+	+	+
C. coli	+	+	+	+	-
C. lari	+	+	+	-	-

Virulence Factor

- Lipopolysaccharides (LPS) with endotoxic activity
- Cytopathic extracellular toxins and enterotoxins have been found

TABLE 36-2 Virulence factors of *Campylobacter* species

Virulence factors	Biological functions
Enterotoxins	Facilitate adherence to the jejunum, ileum, and colon
Lipopolysaccharide	Adhesion
PEB1	Superficial antigen that has been found to be a major adhesion protein
Adhesion proteins	Adhesion
Cytotoxic enzymes	Cytotoxicity action
S protein	Found exclusively in *Campylobacter fetus* and is the major virulence factor. Inhibits C3b binding responsible for both the serum and phagocytic resistance of the bacteria


Environmental reservoir and route of transmission
Clinical manifestation
Consumption of contaminated food or water
Contact with animals
Person-to-person
Brain abscess
Meningitis
Miller-Fisher syndrome
Periodontitis
Guillain-Barré syndrome
Bacteremia*
Septicemia*
Endocarditis*
Myocarditis*
Barrett's esophagus?
Gastro-esophageal reflux?
Hepatitis
Gastritis?
Gastroenteritis (acute and persistent watery diarrhea to dysentery)
IBD?
IBS?
Colorectal cancer?
Reactive arthritis
Bone infection
Soft tissue infection
*Systemic manifestations may be acquired via extraintestinal routes (respiratory, cellulitis, urinary)

Pathogenesis

- The infection by oral route from food, drink, or contact with infected animals or animal products(**Milk, meat products**).
- Susceptible to gastric acid (about 10^4 organisums)

Campylobacter - symptoms

- Incubation: 4-8d
- Acute enteritis: 1w, stools remain positive for 3 w
- Acute colitis
- Acute abdominal pain
- Bacteremia: <1% *C. jejuni*
- Septic abortion
- Reactive arthritis

- diarrhea
- malaise
- fever
- abdominal pain
- usually self-limiting
- antibiotics occassionally
- bacteremia
  - –small minority

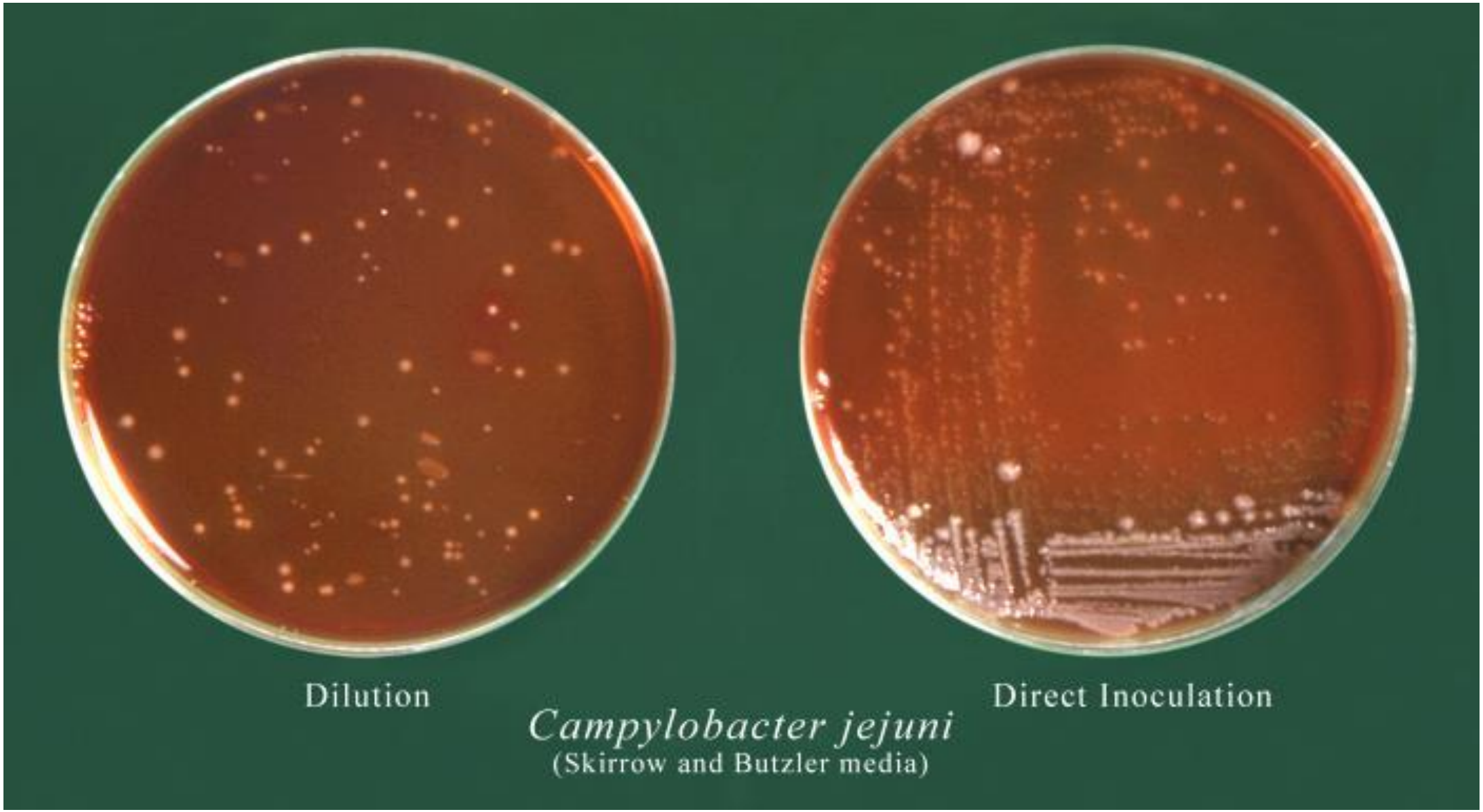
Diagnostic Laboratory Tests

- Specimens: Diarrheal stools
- Smears: Gram-stained smears of stool may show the typical “gull-shaped” rods.

Laboratory Diagnosis

- **Specimen:** Stool (contain mucus, pus and blood), rectal swab, blood

- **Culture:** on selective media containing:
  - -Charcoal: to remove oxygen radicals
  - -Antibiotics: to inhibit other bacteria and enriched with blood
  - -Selective media:
    1) Skirrow's-blood agar
    2) Campy-blood agar


Dilution
Direct Inoculation
Campylobacter jejuni
(Skirrow and Butzler media)

Helicobacter -Taxonomy

kingdom	bacteria
Phylum	proteobacteria
Class	Epsilon Proteobacteria
Order	campylobacterales
Family	Helicobacteraceae
Genus	*Helicobacter*
Species	*H. pylori*
Binomial name	*Helicobacter pylori*

Peptic Ulcer and *Helicobacter pylori* infection:

Helicobacter pylori is a Gram-negative, microaerophilic bacterium found in the stomach.

In 1982, *Barry Marshall* and *Robin Warren*, reported that the microbe is associated with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause.

However, over 80 percent of individuals infected with the bacterium are asymptomatic.

More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract.

Helicobacter pylori

- Curved bacilli –
- Former name - *Campylobacter pylori*,
  - ***H. pylori***

WARREN AND MARSHAL WINS NOBEL PRIZE

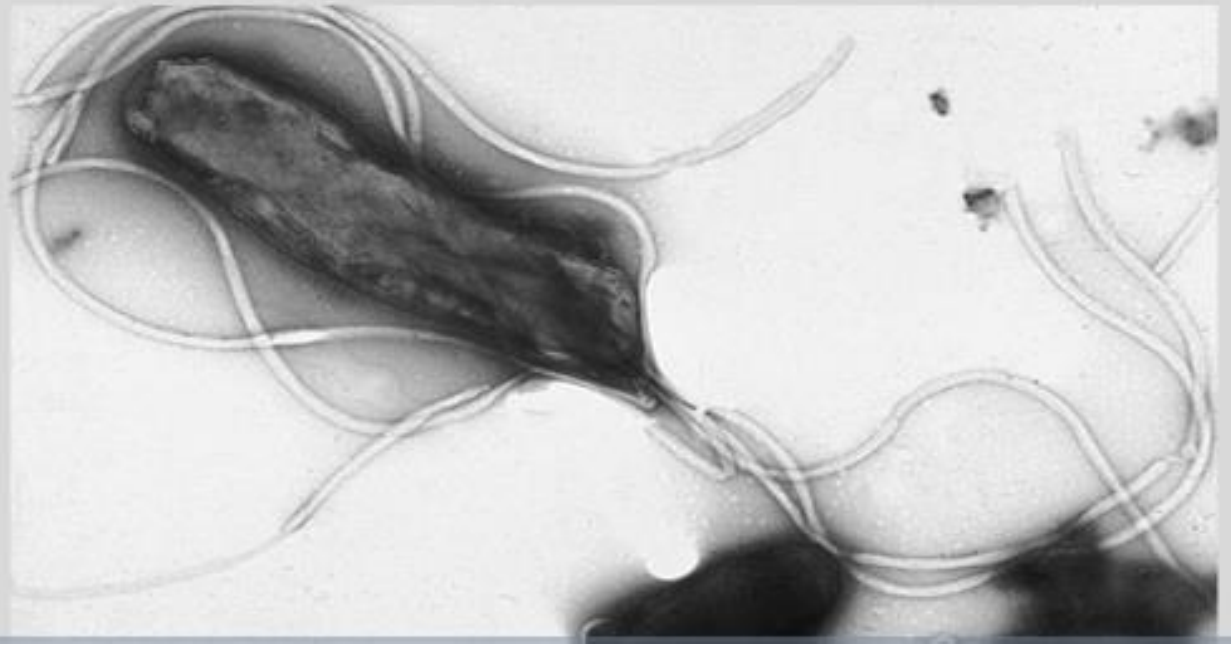
Microscopy:

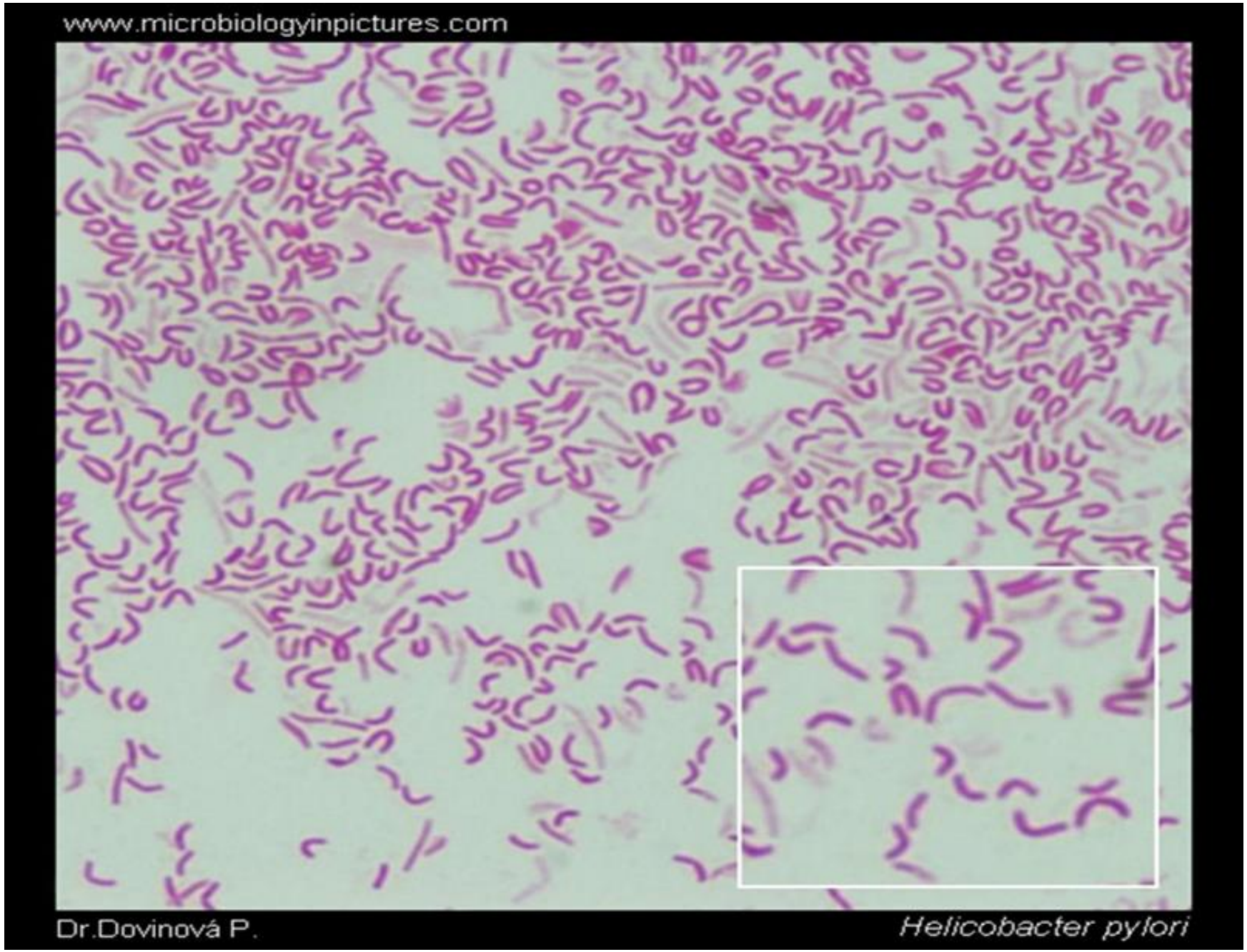
H. pylori is a curved rod, helix-shaped, Gram-negative bacterium measuring about (3 L*0.5 D) micrometers .

All Species are Pleomorphic; Bacillary, spiral, and Coccoidal shape.

All are motile by four to six lophotrichous flagella.

Helicobacter pylori species are non-Spore formers, and most strains have a glycocalyx.




www.microbiologyinpictures.com
Dr.Dovinová P.
Helicobacter pylori

Cultural characteristics:

All *Helicobacter pylori* species are Fastidious bacteria.

The cultivation Enrichment -media:

- Brain heart infusion broth supplemented by fetal calf serum (10%) or/and Bovine serum albumin.
- Brucella broth supplemented by fetal calf serum (10%).

The selective Enriched media:

- Columbia chocolate agar supplemented by 5-10% hemolyzed sheep blood, and specific antibiotics combination (5mg/L trimethoprim, 6mg/L vancomycin, and 6mg/L amphotericin B).

Both Enrichment and Enriched media should be inoculated by the clinical specimens and incubated at **37°C** with a microaerophilic atmosphere (10% CO_2, 5% O_2, and 85% N_2) for up to **8 days** (**humidity** should be **> 95%**).

Colony morphology:

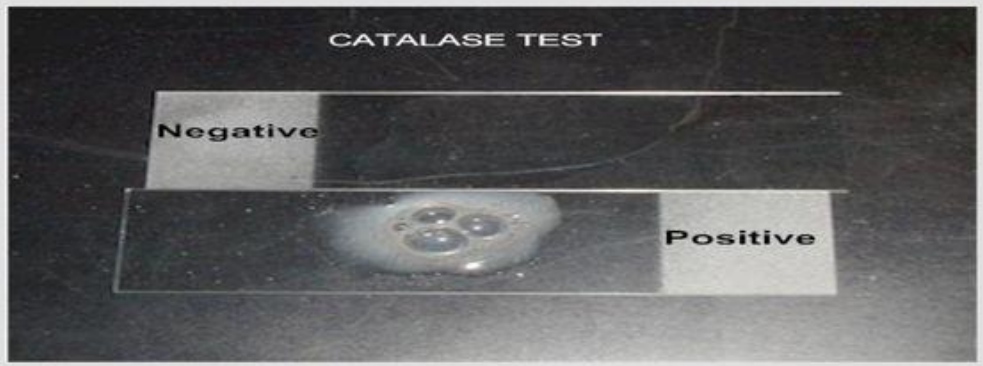
-*H. pylori* species produce a specific colonies with the following characteristics :

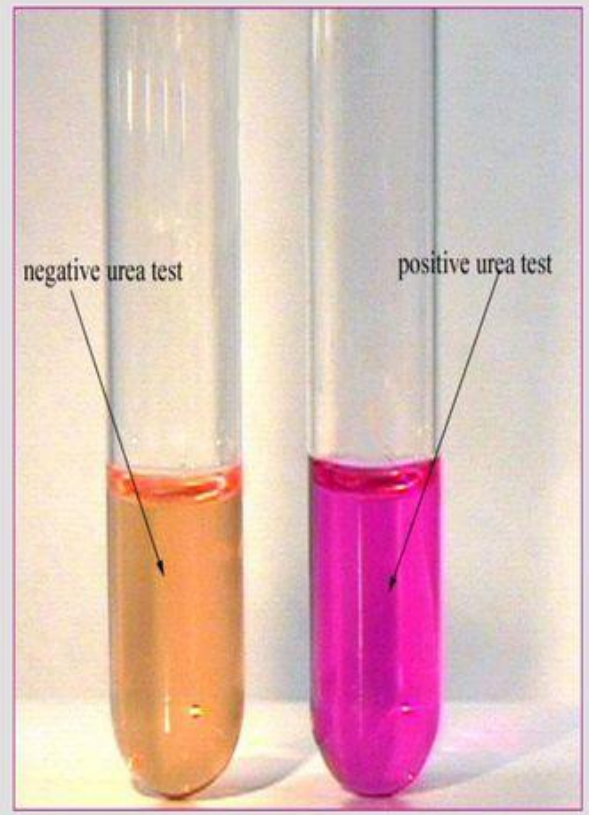
small (0.5-2mm), round, convex, translucent, with enteric margin and non-hemolytic activity.

Biochemical identification:

All *Helicobacter pylori* species show immediate positive oxidase , catalase, and urease (++++) reactions.

All species are also demonstrating alkaline phosphatase, gamma – glutamyl aminopeptidase , and ornithine decarboxylase activities.





Helicobacter pylori Virulence factors:

- **Outer membrane proteins**:

  1-The adhesins protein : adhesion to host cell.

  2- Porins proteins, iron transporters, and Flagella proteins.

- **The Lipopolysaccharide** (LPS)- **O** antigen:

  mimic Lewis blood group antigen found on the gastric epithelium

- **The Exotoxins**: The **Vacuolating** (**Vac A**) toxins:

  : gastric mucosal injury.

- **The Secretory Enzymes**:

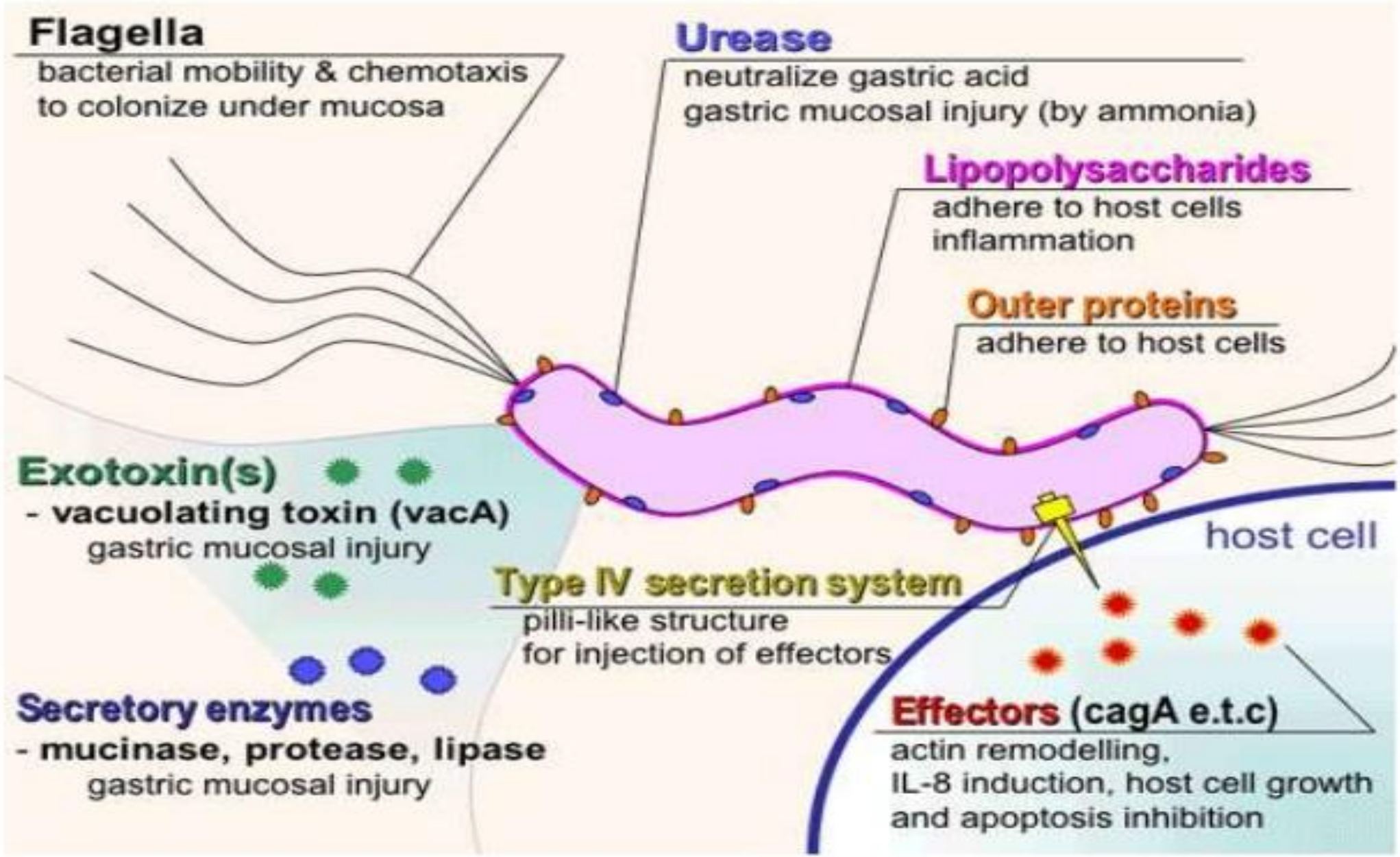
  1- The **Urease**: Neutralize gastric acid, and mucosal injury.

  2- The **mucinase**, lipase, protease: mucosal injury.

- **The Flagella** : motility.

- **The effector cytotoxin** : The cytotoxin associated gene A (**Cag A**):

  cause uncontrolled host cell growth and **apoptosis inhibition**.


Flagella
bacterial mobility & chemotaxis
to colonize under mucosa
Urease
neutralize gastric acid
gastric mucosal injury (by ammonia)
Lipopolysaccharides
adhere to host cells
inflammation
Outer proteins
adhere to host cells
Exotoxin(s)
- vacuolating toxin (vacA)
gastric mucosal injury
Type IV secretion system
pilli-like structure
for injection of effectors
host cell
Secretory enzymes
- mucinase, protease, lipase
gastric mucosal injury
Effectors (cagA e.t.c)
actin remodelling,
IL-8 induction, host cell growth
and apoptosis inhibition

Pathogenesis and clinical picture:

Transmission : **1- Person to Person** (Oral-Oral or Fecal-Oral).

2- Waterborne mainly within People living in crowded condition, inadequate sanitation practices, and poor hygiene.

3-Iatrogenic transmission:

mediated by contaminated Endoscopy.

1-Primary infection:

-*H. pylori* enters the stomach.

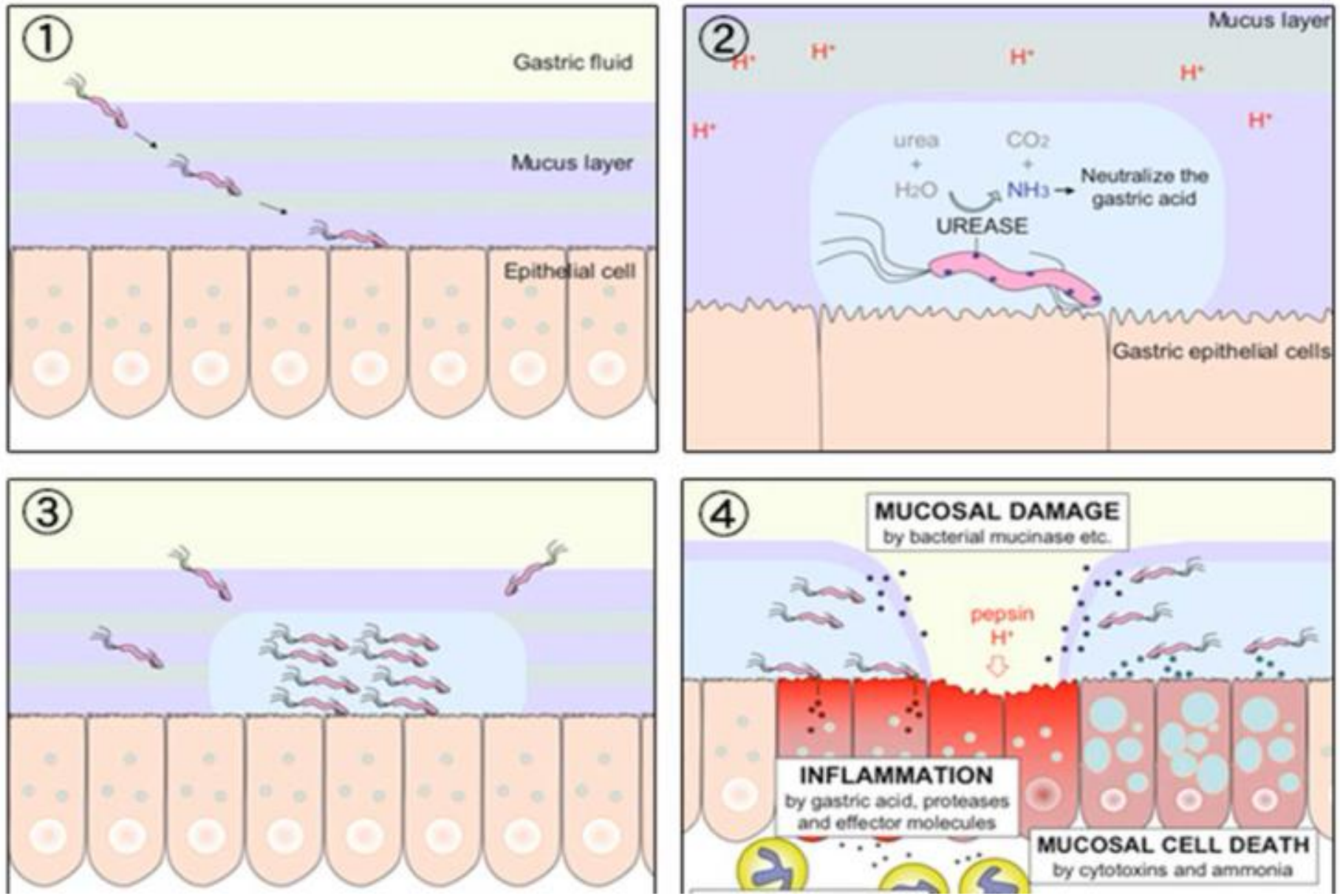
- To avoid being carried into the lumen, *H. pylori* senses the pH-gradient and swims away from the acidic microenvironment.

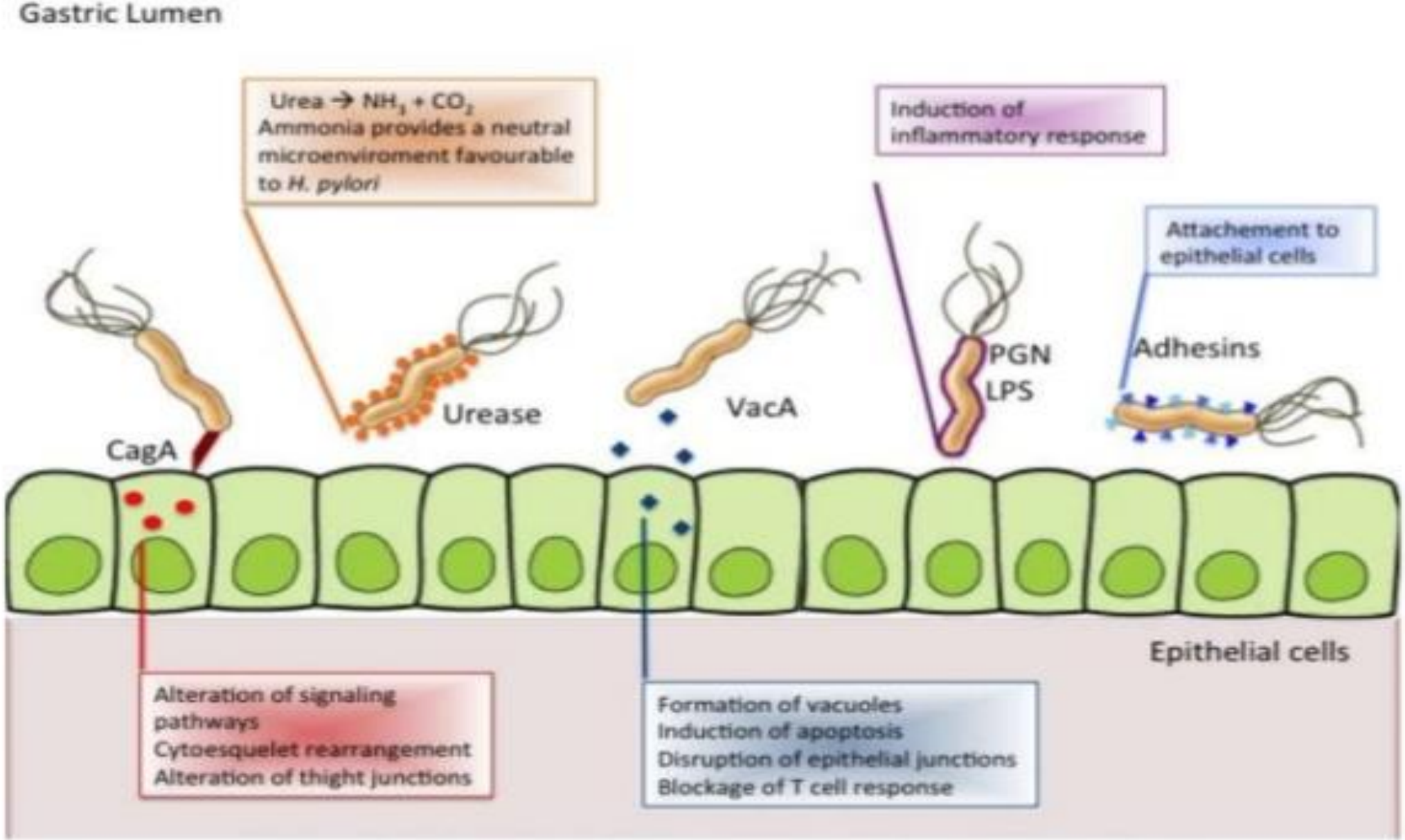
- The microbe invades the mucus layer .

- Ammonia will neutralize gastric acid and damage the epithelial cells of gastric mucosa.
- The microbial toxins will also destroy epithelial mucosa.
- Colonization of gastric mucosa stimulates chemotaxis of phagocytes, lymphocytes and cellular immunity.
- **Antrum Gastritis** (acute infection).
- **Corpus Gastritis** (acute infection).
- Both types of gastritis could lead to **gastric ulceration**. (gastric acid concentration reduced)
- **Duodenal ulceration :** (mainly chronic infection)**:** (gastric acid concentration increased).
- **Duodenal atrophy:** (chronic infection): (gastric acid concentration decreased).

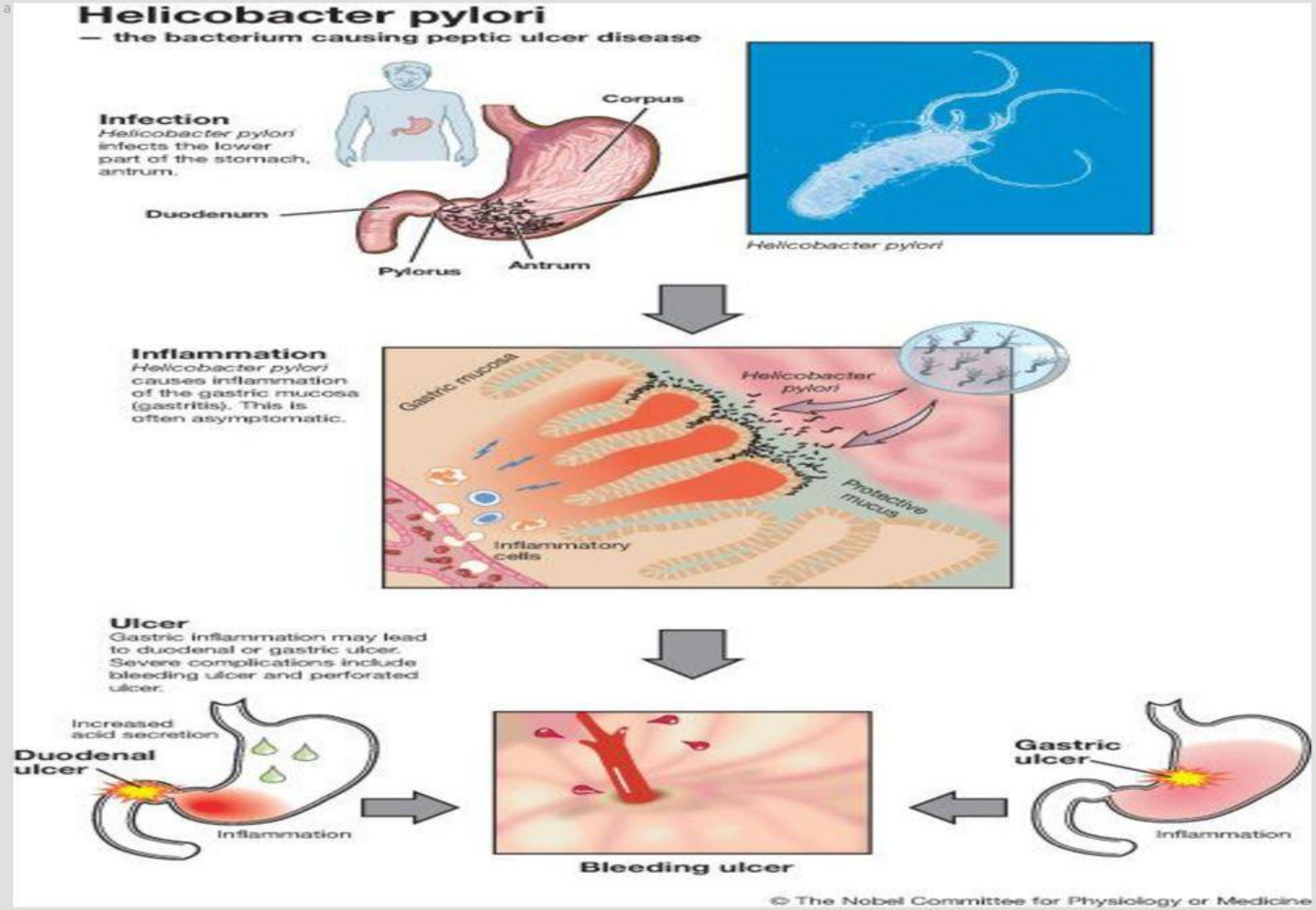
- The risk of **stomach cancer** increased by chronic infection.

2- Secondary infection: Latency.




Gastric Lumen
Urea → NH_3 + CO_2
Ammonia provides a neutral microenviroment favourable to *H. pylori*
Induction of inflammatory response
Attachement to epithelial cells
PGN
LPS
Adhesins
Urease
VacA
CagA
Epithelial cells
Alteration of signaling pathways
Cytoesquelet rearrangement
Alteration of thight junctions
Formation of vacuoles
Induction of apoptosis
Disruption of epithelial junctions
Blockage of T cell response

Pathogenesis and clinical picture:



The secondary and chronic infections caused by **Toxigenic *Helicobacter pylori* strains** (Cag A +++) could be complicated by:

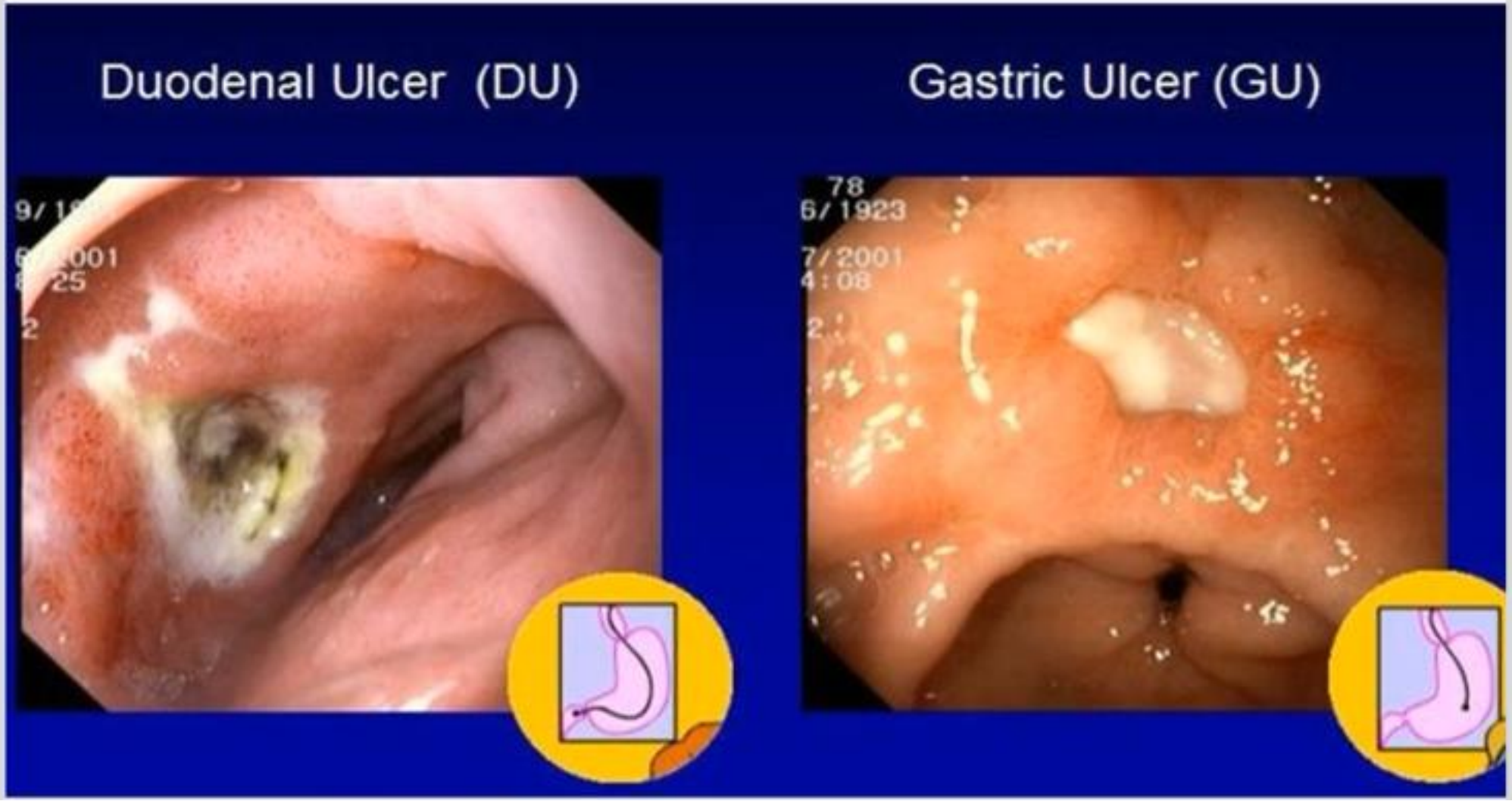
1- Adenocarcinoma of the stomach.

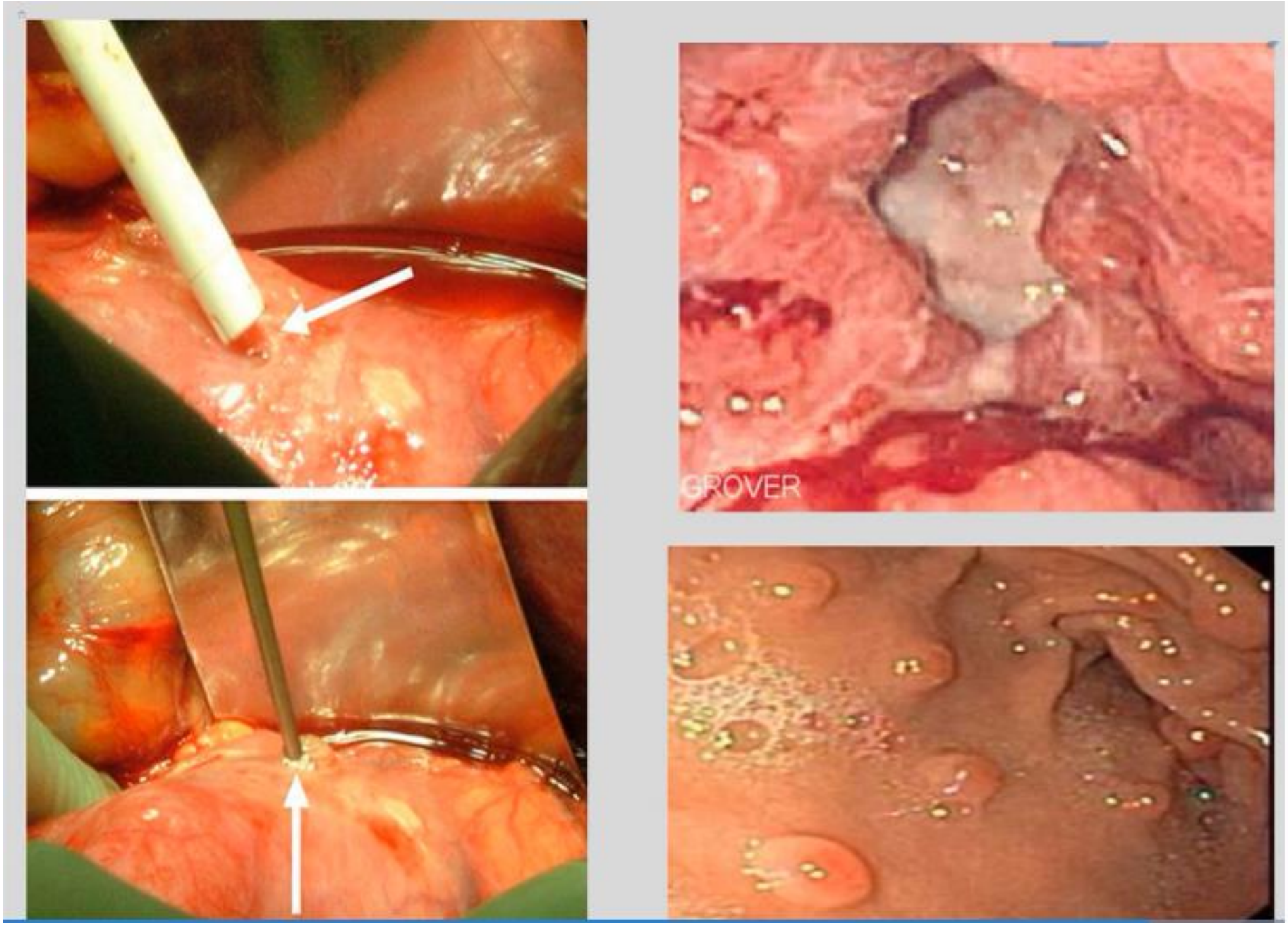
2- MALToma of the stomach:

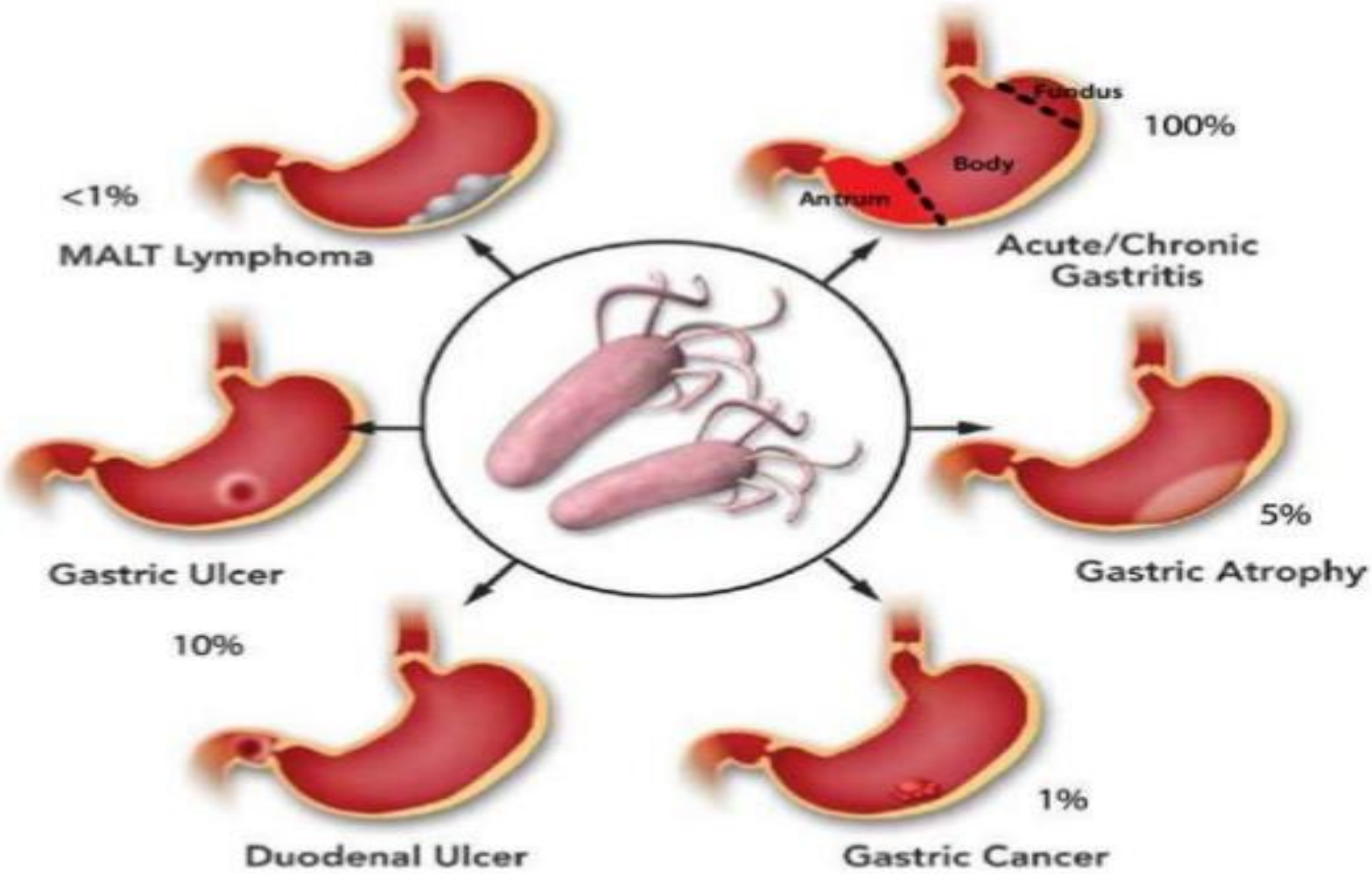
It is a rare type of Non-Hodgkin's lymphoma of the stomach (**B-cell Lymphoma**).

Chronic ulceration of stomach and duodenum could be converted into **Bleeding ulceration**.

Clinical picture of *H. pylori*:




GROVER


<1%
MALT Lymphoma
Fundus
Body
Antrum
100%
Acute/Chronic Gastritis
Gastric Ulcer
10%
5%
Gastric Atrophy
Duodenal Ulcer
1%
Gastric Cancer

H. pylori diagnostic tests	
Invasive	**Non-invasive**
Gastroscopy • standard videoendoscopy • high magnifying endoscopy • chromoendoscopy	**Serology** • near patient tests (HelicoTest) • ELISA
Rapid urease test (CLO test)	**UBT test C13, C14**
Histology (Giemsa staining)	**HP stool antigen test (HPSA)** • polyclonal antibody–based ELISA • monoclonal antibody –based ELISA
Microbiology Culture	**Gastropanel** • *H. pylori* antibodies • Pepsinogen I, II • Gastrin 17

Serology

(Qualitative Or Quantitative Ig G)

Advantages

- Widely available
- Inexpensive
- Good NPV

Disadvantages

- Poor PPV if Hp prevalence is low
- Not useful after treatment

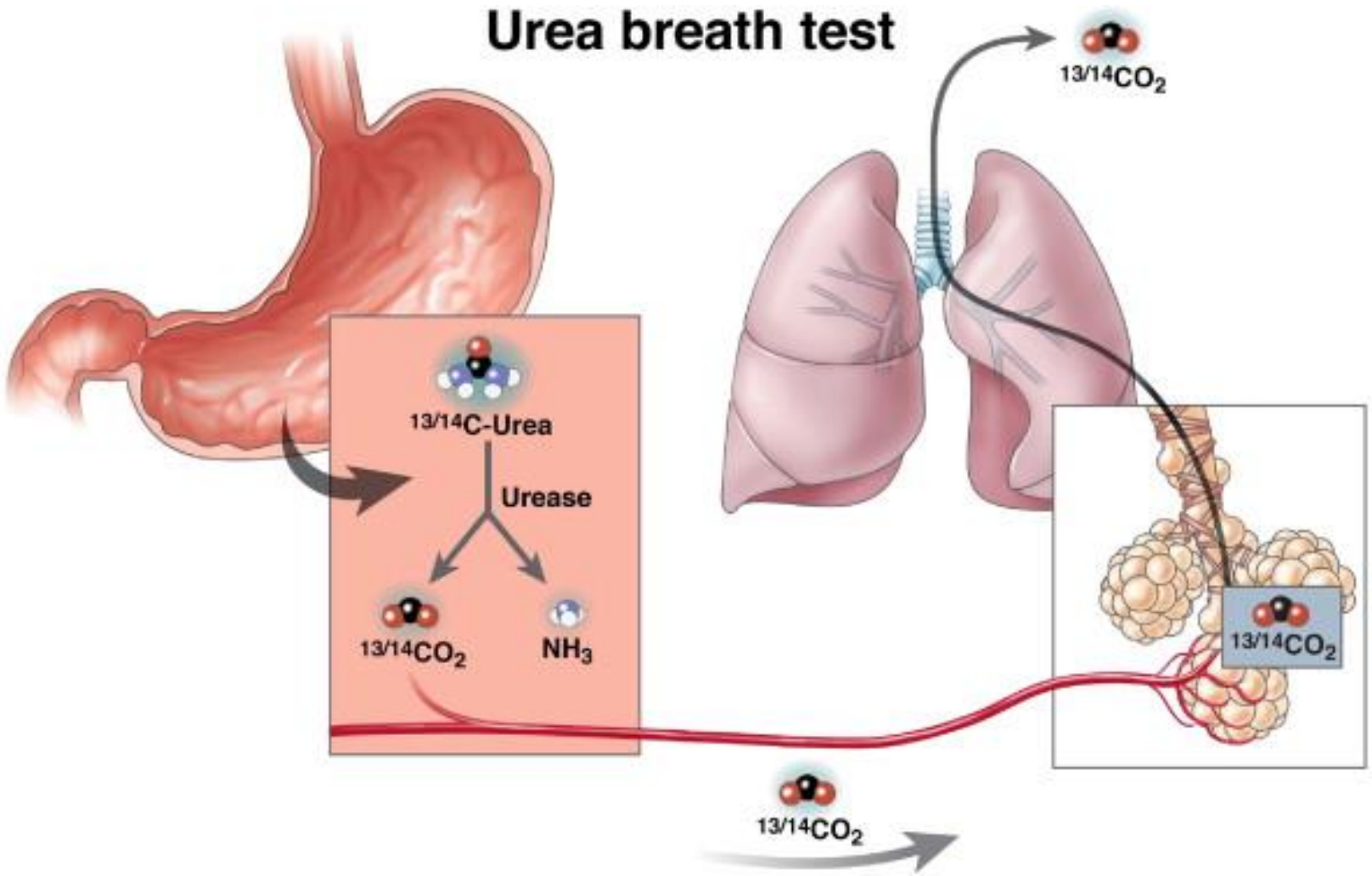
Urea Breath Test

Advantages

- Identifies active infection
- Accuracy (PPV, NPV) not affected by Hp prevalence
- Useful both before and after treatment

Disadvantages

- Availability and reimbursement inconsistent
- Accuracy affected by PPI and antibiotic use
- Small radiation dose with 14C test



1. Patient swallows a labeled C13/14 urea tablet. Dissolves to release ^{14}C-urea.
2. If present, *H. pylori* metabolizes ^{14}C-urea to labeled carbon dioxide ($^{14}CO_2$) and ammonia via the enzyme urease.

$$H_2N(^{13/14}CO)NH_2 + H_2O \rightarrow \text{urease} \rightarrow 2NH_3 + {}^{13/14}CO_2$$

3. $^{14}CO_2$ is transported in the blood to the lungs.
4. Patient exhales. $^{14}CO_2$ is captured for analysis.

Stool Antigen Test

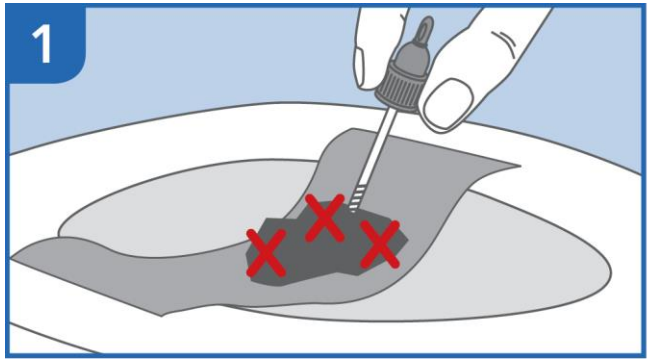
Advantages

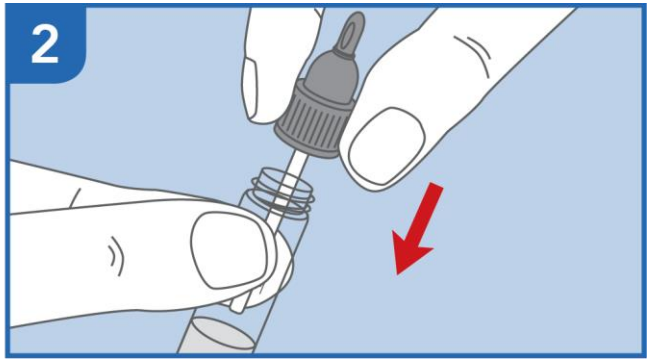
- Identifies active infection
- Accuracy (PPV, NPV) not affected by Hp prevalence
- Useful both before and after treatment (monoclonal test)

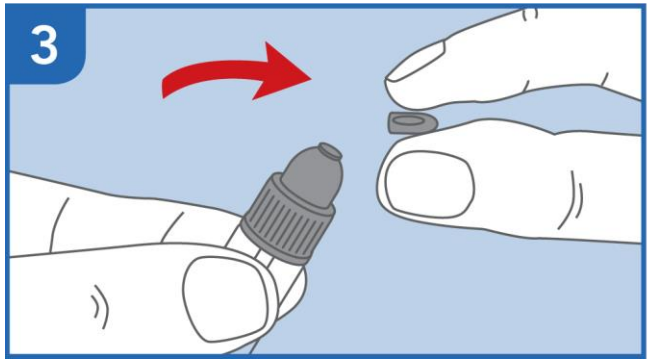
Disadvantages

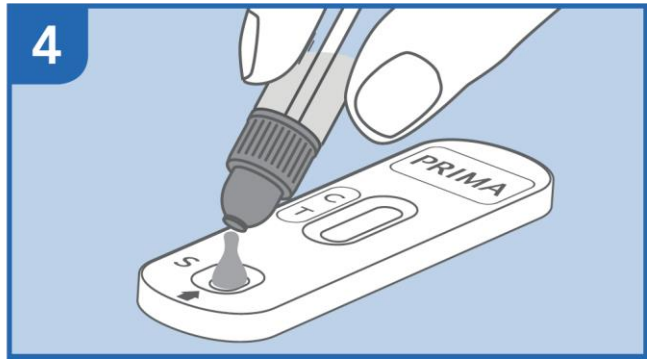
- Fewer data available for polyclonal test
- Accuracy affected by PPI and antibiotic use

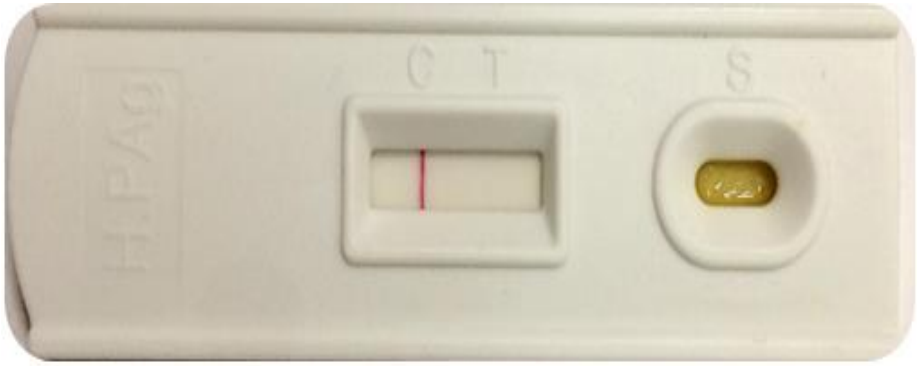
Helicobacter pylori - Stool Antigen Test

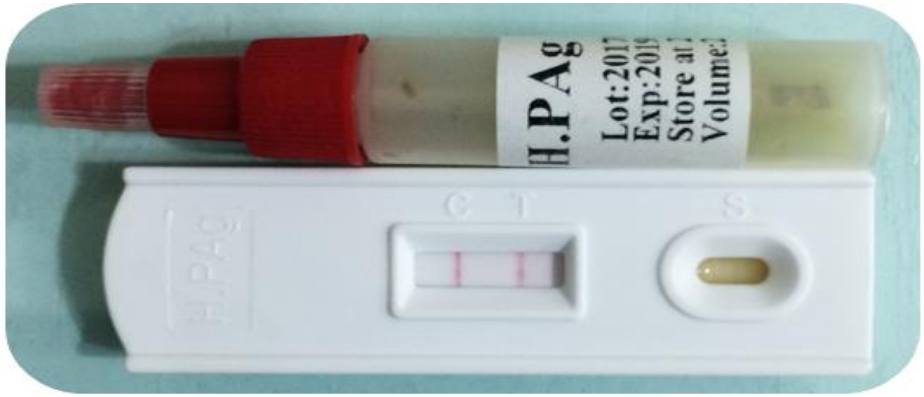












Rapid Urease Test

Advantages

- Rapid results
- Accurate in patients not using PPIs or antibiotics
- No added Pathology cost

Disadvantages

- Requires endoscopy
- Less accurate after treatment or in patients using PPIs

CLO (Campylobacter-like organism) test

Histology

Advantages	Disadvantages
• Excellent sensitivity and specificity, especially with special and immune stains • Provides additional information about gastric mucosa • Asses premalignant lesions	• Expensive (endoscopy and Pathology costs) • Some inter observer variability • Accuracy affected by PPI and antibiotic use

Cultures

Advantages	Disadvantages
• Specificity ≈ 100% • Allows antibiotic sensitivity testing	• Requires endoscopy • Less accurate after treatment or in patients using PPIs

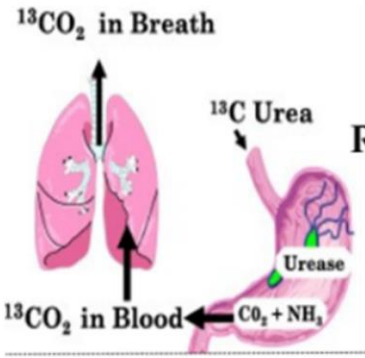
PCR Assay

Advantages	Disadvantages
• Excellent sensitivity and specificity • **Permits detection of antibiotic resistance**	• Not widely available • Technique not standardized • Expensive

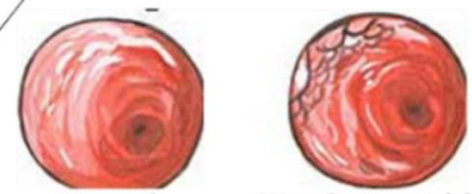
Non-Invasive methods

Invasive methods

Current diagnostic tests for *H. pylori*



Respiratory tests urea breath test
(^{13}C-UBT, ^{14}C-UBT)


Normal

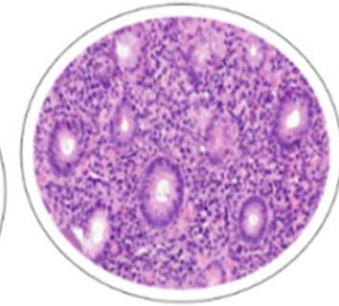
H. pylori gastritis

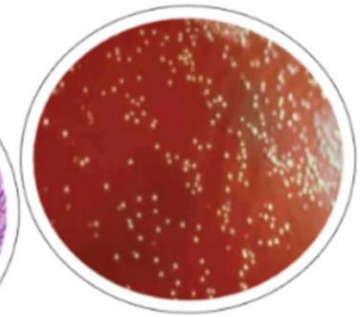
Endoscopy

Culture

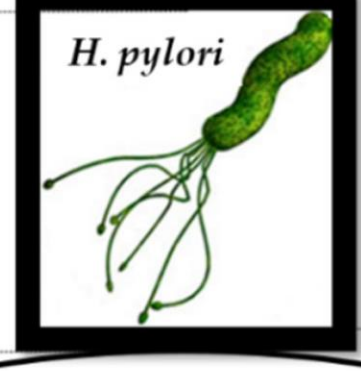
Histology

Rapid urease test





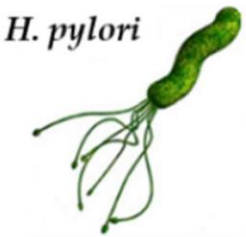
Stool antigen (SAT)

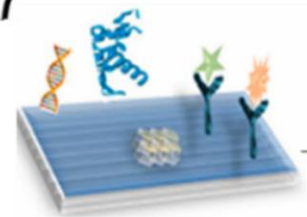


Serology

Biosensors methods

PCR Test





- Optoelectronic nano-biosensors
- Piezoelectric sensors
- Electrochemical biosensors

Detection

Standard Dosages Used In Most Of The Treatment Regimens

Antibiotics

Clarithromycin 500 Mg,
Amoxicillin1 G,
Metronidazole 500 Mg,
Tinidazole 500 Mg,
Tetracycline 500 Mg,
Levofloxacin 500 Mg,
Rifabutin 300 Mg.

PPIs

- Omeprazole20 Mg, Lansoprazole 30 Mg, Pantoprazole 40 Mg, Rabeprazole20 Mg, Esomeprazole 40 Mg, Or Dexlanzoprazole 30 Mg.
- Bismuth Subsalicylate 524 Mg
- Bismuthsubcitrate 420 Mg