The Vibrio

General characters

1- They are thin, curved, comma shaped Gram-negative, rigid and actively motile (polar flagellum) bacilli.

2- They are non-lactose fermenting and oxidase positive (differentiated from enterobacteriaceae).

3- Grow at alkaline pH 9. No known pathogen can grow at this pH (Alkaline pH used in selective media like TCBS).

4- Active motile producing dancing motility due to the single polar flagellum

5- They ferment glucose with the production of acid only. (Can be differentiated from Pseudomonas which G –ve bacilli (not curved) and sugar non fermenter (TSI=K/K)

6- All bacteria have one circular chromosome, while V.cholerae has two

7- This bacterium has two forms: an infective form which responsible for epidemic form of cholera and can be isolated using culture media, viable but nonculturable form which is coccoid form and present in aquatic environment but cannot be culture and act as a more resistant form of bacteria and responsible for new epidemics.

Medically important vibrio are shown in table 1

Vibrio cholerae

Morphology and Identification

***V cholerae* is a comma-shaped, curved rod 2–4 µm long in first isolation but (Figure 1) upon prolonged cultivation, may become straight rods that resemble gram-negative enteric bacteria.**

It shows darting type of motility due to the single polar flagellum (Figure 2).

Susceptible to the compound O/129 disc (2,4-diamino- 6,7-diisopropylpteridine phosphate), which differentiates them from *Aeromonas* species, which are resistant to O/129.

Fig.1. Gram negative comma or curved bacilli of *V.cholerae*

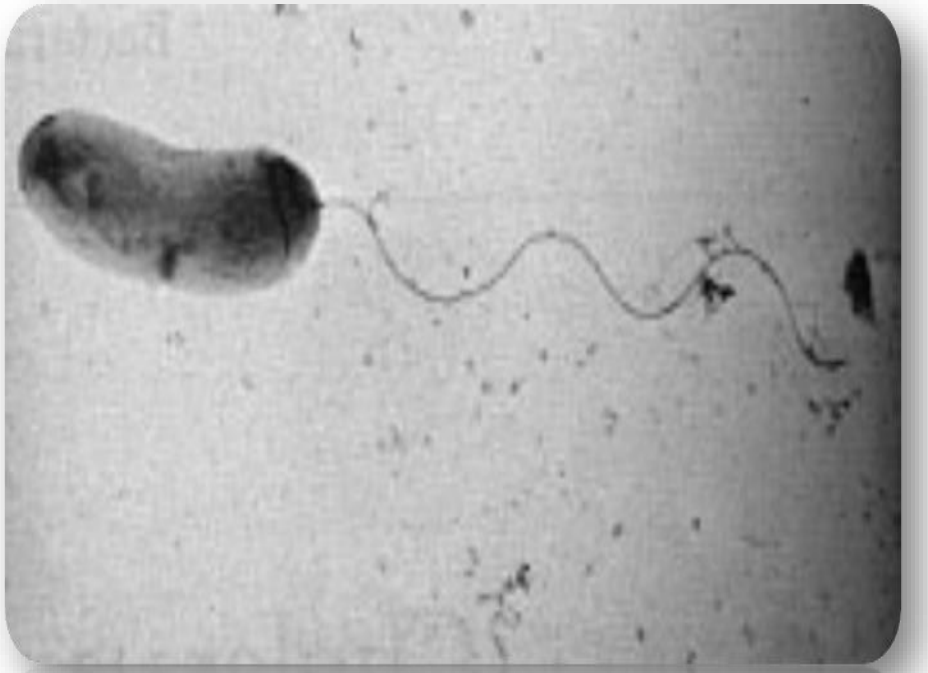

Fig.2. Lophotrichous V.cholerae

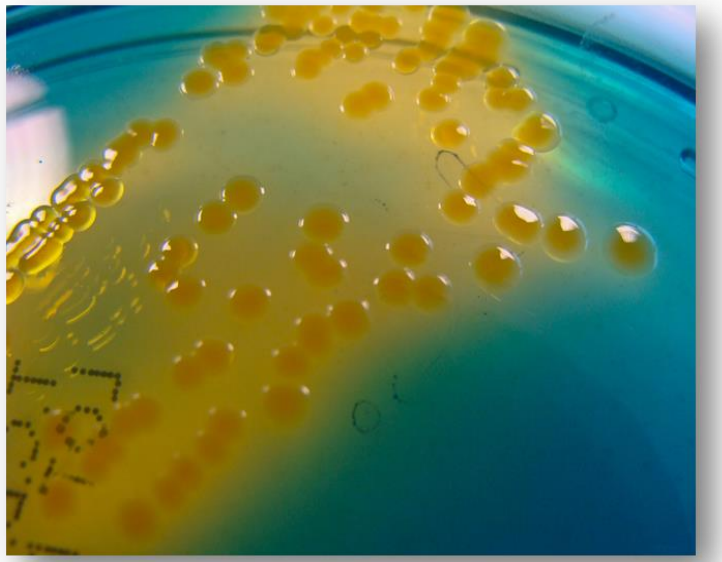

Fig.3. Gram negative comma or curved bacilli of *V.cholerae*

TABLE [illegible]–1 The Medically Important Vibrios

Organism BHU	Human Disease
Vibrio cholerae serogroups O1 and O139	Epidemic and pandemic cholera
Vibrio cholerae serogroups non-O1/non-O139	Cholera-like diarrhea; mild diarrhea; rarely, extraintestinal infection
Vibrio parahaemolyticus	Gastroenteritis, perhaps extraintestinal infection
Others *Vibrio mimicus, Vibrio vulnificus, Vibrio hollisae, Vibrio fluvialis, Vibrio damsela, Vibrio anginolyticus, Vibrio metschnikovii, Vibrio cincinnatiensis*	Ear, wound, soft tissue, and other extraintestinal infections (all uncommon)

Cultural characteristics

V. Cholerae grows well on **thiosulfate-citrate-bile-sucrose (TCBS)** agar, a media selective for vibrios, on which it produces yellow colonies (**sucrose fermented**) (Figure 3). Oxidase positive. Non lactose fermenters on Mac Conkey agar.

In areas where cholera is endemic, enrichment cultures in **alkaline peptone water** for 6 hrs then subculture on TCBS is recommended.

Antigenic Structure and Biologic Classification

There are at least 200 O antigen groups. *V cholerae* strains of O group 1 and O group 139 (Bengal) cause classic cholera; occasionally, non-O1/non-O139 *V cholerae* causes cholera-like disease. The *V cholerae* serogroup O1 antigen has three serotypes: Ogawa, Inaba, and Hikojima and Two biotypes: classic and El Tor. The El Tor biotype produces a hemolysin, gives positive results on the Voges-Proskauer test, and is resistant to polymyxin B.

V cholerae O139 is very similar to *V cholerae* O1 El Tor biotype. *V cholerae* O139 does not produce the O1 lipopolysaccharide and have capsule (V.cholera O1 group doesn't make capsule).

Virulence factors

1- Pili

2- cholera toxin

V cholerae produce a heat-labile enterotoxin consisting of subunits A and B(5). Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A1 yields increased levels of intracellular cyclic adenosine monophosphate (cAMP) and results in prolonged hypersecretion of water and electrolytes (Figure 4). Electrolyte-rich diarrhea (rice water) occurs as much as 20–30 L/day—with resulting dehydration, shock, acidosis, and death. The genes for *V cholerae* enterotoxin are on the bacterial chromosome. Cholera enterotoxin is antigenically related to LT of *Escherichia coli.*

Person with normal gastric acidity may have to ingest as many as 10^{10} or more *V cholerae* to become infected when the vehicle is water because the organisms are susceptible to acid. When the vehicle is food, as few as 10^2–10^4 organisms are necessary because of the buffering capacity of food. Any medication or condition that decreases stomach acidity makes a person more susceptible to infection with *V cholerae.*

Cholera is not invasive (no bacteremia) disease just localized to the GIT (small intestine) without inflammation

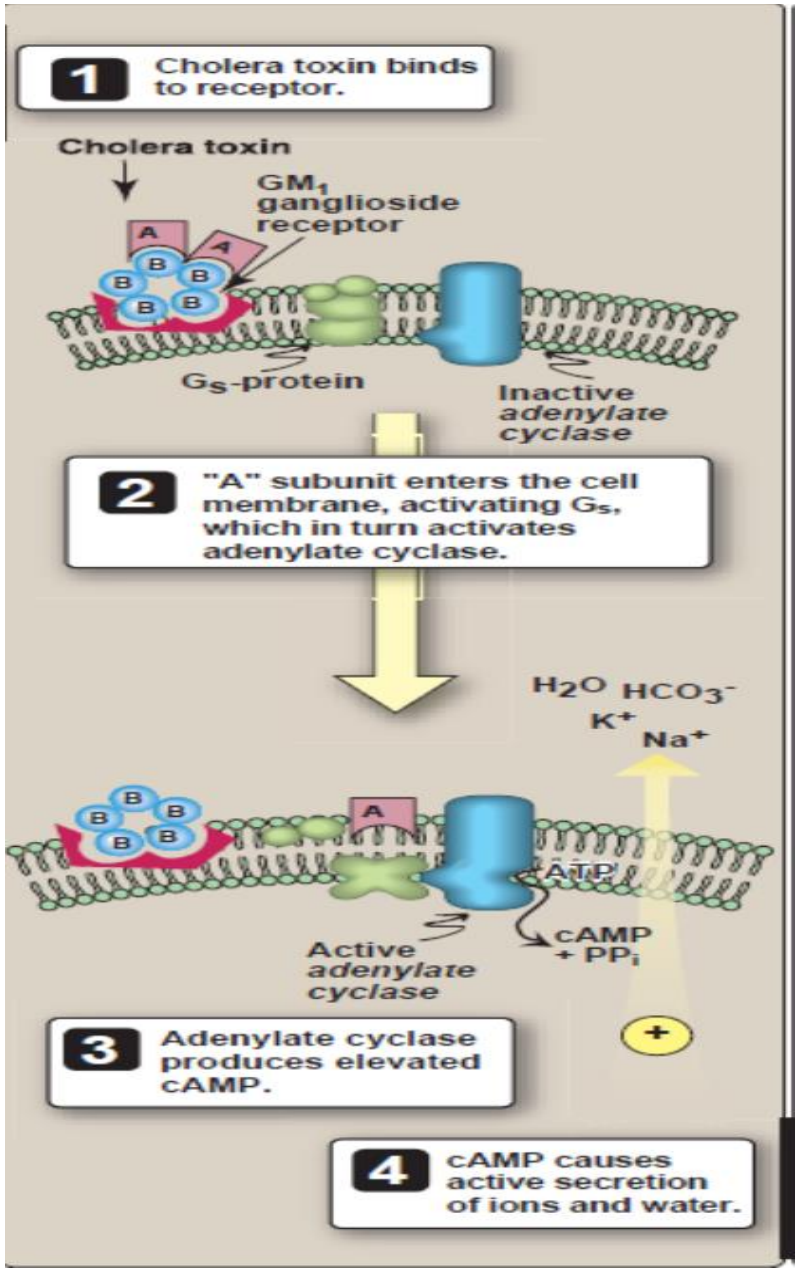

Fig.4. Gram negative comma or curved bacilli of *V.cholerae*

Clinical Findings

The incubation period is 12 hours to 3 days.
There is a sudden onset of nausea and vomiting and profuse diarrhea with abdominal cramps.
Stools, which resemble “rice water,” contain mucus, epithelial cells, and large numbers of vibrios. There is rapid loss of fluid and electrolytes, which leads to profound dehydration, circulatory collapse, and anuria. There is no bacteremia.

Laboratory diagnosis

Fresh stool collected in dry and clean containers with proof and tightly closed (Screw cups) or **rectal swabs**

Direct microscopic examination:
- Hanging drop preparation shows darting type motility.
- Gram-staining shows them to be Gram negative and comma shaped.

Culture:
Pre-enriched technique using alkaline peptone water for 6-8 hrs then subculture on TCBS. Colonies are sucrose fermenters (yellow pigmentation). It shows oxidase positive, nitrate reduction positive, fermentation of glucose, sucrose, mannose and arabinose, cholera red reaction positive, indole positive and slide agglutination with O group polyvalent or mono specific sera differentiates it into Ogawa, Inaba and Hikojima types.

Treatment

The most important part of therapy consists of water and electrolyte replacement.
Oral tetracycline and doxycycline tend to reduce stool output in cholera and shorten the period of excretion of vibrios.

Control rests on education and on improvement of sanitation, particularly of food and water. Patients should be isolated, their excreta disinfected, and contacts followed up.
Chemoprophylaxis with antimicrobial drugs may have a place. Repeated injection of a vaccine containing either lipopolysaccharides extracted from vibrios or dense *Vibrio* suspensions can confer limited protection to heavily exposed persons (eg, family contacts) but is not effective as an epidemic control measure.

V.parahemolyticus

It is a halophilic Vibrio that cause acute gastroenteritis after ingestion of contaminated sea-food such raw fish or shellfish.

Clinically characterized by nausea, vomiting, abdominal cramps, fever and watery to bloody diarrhea. Fecal leukocytes are often present.

Can grow on TCBS media but without fermentation of sucrose (green colonies), oxidase positive and morphologically similar to V.chlerae.

Other Vibrios like V.vulnificus can cause: wound infection, Bacteremia, and gastroenteritis

Treatment: Tetracycline or Ciprofloxacin

Aeromonas hydrophila

General characteristics

- Gram –ve bacilli similar to enteric bacteria
- Motile
- Hemolytic
- Can grow on Mac Conkey agar (confused with enteric bacteria)
- Oxidase positive
- Resistant to compound O/129 (Vibrio sensitive)
- Can not grow in media containing 6.5% NaCl (Vibrio can grow)
- No suitable animal model that reproduce human aeromonas associated diarrhea (So Koch's postulates not satisfied)
- Can cause bacteremia, wound infection (trauma occurs in water environment)
- Susceptible to tetracycline, aminoglycosides and 3rd generations of cephalosporins Like Claforan (Cefotaxime and Suprax (Cefixime).

Plesiomonas shigelloides

General characteristics

- Gram –ve bacilli
- Oxidase positive
- Isolates from fresh water fish and many mammals
- Most isolates from humans have been from stool culture od patients with diarrhea.
- Can grow on differential media used to isolate salmonella and Shigella from stool samples and some strains have antigen with Shigella sonnei (cross reaction-false positive result).
- Oxidase positive (Shigella sp are oxidase negative)

Campylobacter spp.
***C.Jejuni and C.coli* (common causes of food poisoning from poultry animal products.**

Morphology and identification

- ❑ Gram negative with comma, S or gull wing shapes (Figure 5).
- ❑ Motile with a single polar flagellum

Culture

- ❑ Requires selective media such as Skirrows media (Vancomycin, Polymyxin B, Trimethoprim, Amphotericin B) or Sodium pyruvate, Vancomycin, Cefoperazone, cycloheximide) to inhibit other bacteria and fungi.
- ❑ Needs 5-10% of Co2 (Campy gas generating pack)
- ❑ For primarily isolatesion,42C is used
- ❑ Incubation time 48-72 hours
- ❑ Colonies are colorless to gray, round and convex (Figure 6)

Fig.5. Gram –ve comma, S or gull wing shapes of C.jejuni

Biochemical reactions

- ❖ **Oxidase +ve**
- ❖ **Catalase +ve**
- ❖ **Don’t oxidize or ferment CHO**
- ❖ **Reduce Nitrate**
- ❖ **Produce H2S**
- ❖ **Hippurate test**

Clinically characterized by abdominal cramps, profuse diarrhea that may be grossly bloody, headache, malaise and fever. Sometime when the abdominal pain periumblical confused with appendicitis. The illness is self limited to a period of a week and occasionally take longer. Most cases resolve without drugs but erythromycin can shorten the duration of Fecal shedding of bacteria.

Guillian Barre syndrome: this is autoimmune disease occurs after infection with certain strains of C. jejuni gastroenteritis or with other viruses that infect respiratory system like EBV. It is due to molecular mimicry between pathogen structures and body tissue so immune reaction will attack body tissue like myelin sheath of motor nerve causing demyelination (paralysis).

Reiter's Syndrome is a reactive arthritis that develops in response to an infection (Campylobacter, Salmonella, Shigella, Yersina) and characterized by a triad of arthritis, conjunctivitis, and nonspecific urethritis.

Laboratory diagnosis

Stool: Gram stained smears reveal Gram negative S or gull wing appearance. Motility test: darting motility like Vibrio

Culture on selective media like Skirrow media at 42C. For 48-72 hrs under microaerophilic conditions

Gram stain to see characteristic shapes and motility test

Do biochemical reactions and susceptibility like table 1

Table .1. characteristics of C.jejuni

Characteristics of C.jejuni

Characteristic	Result
Growth at 25 °C	-
Growth at 35-37 °C	-
Growth at 42 °C	+
Nitrate reduction	+
Catalase test	+
Oxidase test	+
Growth on MacConkey agar	+
Motility (wet mount)	+
Glucose utilization	-
Hippurate hydrolysis	+
Resistance to nalidixic acid	-
Resistance to cephalothin	+

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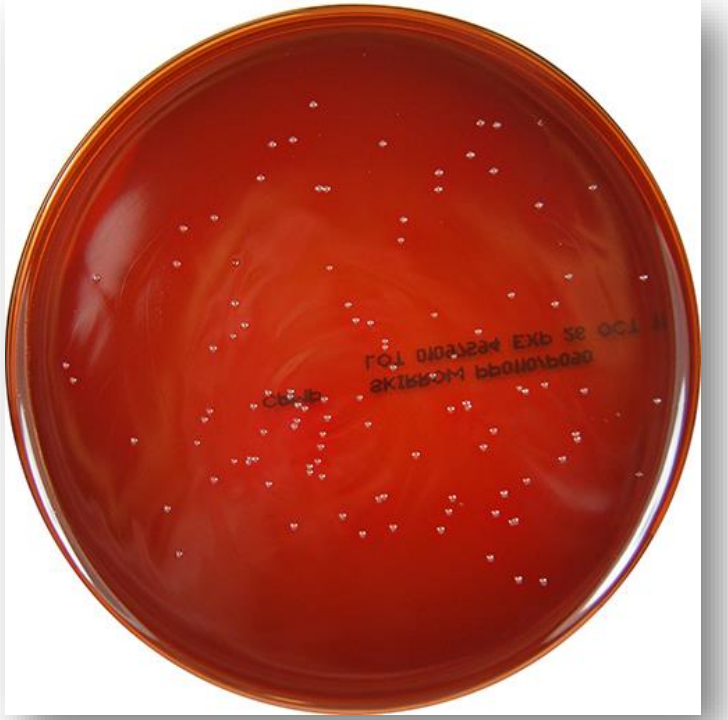


Fig.6.Growth of C.jejuni on blood agar

Helicobacter pylori

H pylori is a spiral-shaped gram-negative rod. *H pylori* is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric cancer (the first bacteria to be documented announced by WHO that can cause cancer.

Morphology and Identification

Typical Organisms

H pylori has many characteristics in common with campylobacters. It has multiple flagella at one pole and is actively motile.

Culture

Gastric biopsy requires for isolation and *H pylori* grows in 3–6 days when incubated at 37°C in a microaerophilic environment, as for *C jejuni*. The media for primary isolation include Skirrow's medium with vancomycin, polymyxin B, and trimethoprim, chocolate medium, and other selective media with antibiotics (eg, vancomycin, nalidixic acid, amphotericin). The colonies are translucent and 1–2 mm in diameter (Figure 7).

Growth Characteristics

H pylori is oxidase positive and catalase positive, has a characteristic morphology, is motile, and is a strong producer of urease.

Fig.7.Colonies of H.pylori on blood agar

Pathogenesis and Pathology

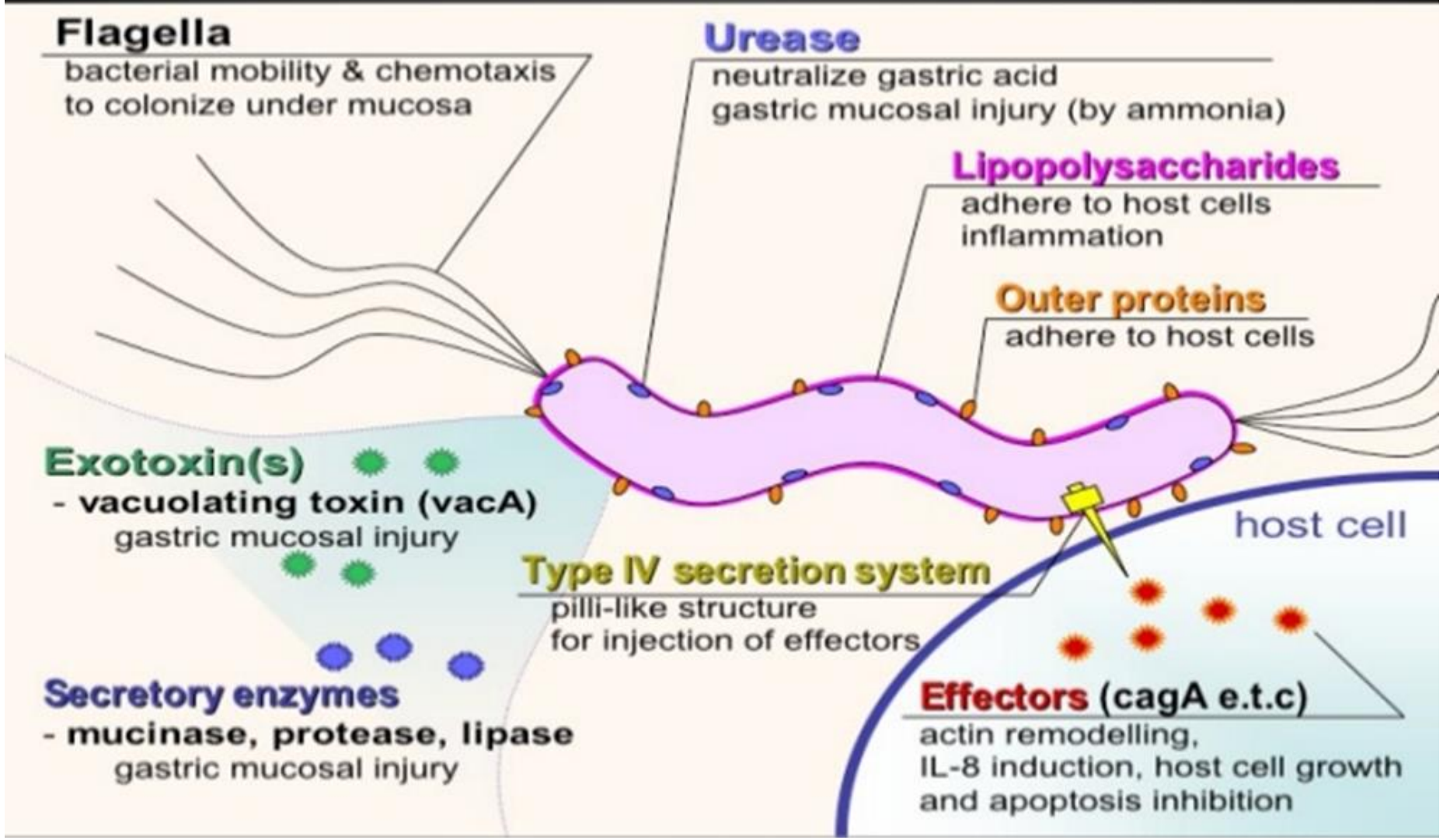
H pylori grows optimally at a pH of 6.0–7.0 and would be killed or not grow at the pH within the gastric lumen. Gastric mucus is relatively impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1.0–2.0); on the epithelial side, the pH is about 7.4. *H pylori* is found deep in the mucous layer near the epithelial surface where physiologic pH is present. *H pylori* also produces a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus. *H pylori* produces potent urease activity, which yields production of ammonia and further buffering of acid. Virulent strains produce two types of exotoxins like Vac A (vacoulating) (cause gastric epithelial injury and Cag A (cytotoxic assoiated gene) (inhibit cell apoptosis (Figure 8). *H pylori* is quite motile, even in mucus, and is able to find its way to the epithelial surface.

The exact mechanism of mucosal inflammation and damage are not well defined but probably involve both bacterial and host factors. Bacterial factors are those shown in figure 8.

Histologically, gastritis is characterized by acute and chronic inflammation. Polymorphonuclear and mononuclear cell infiltrates are seen within the epithelium and lamina propria.

About 90% of patients with duodenal ulcers and 50–80% of those with gastric ulcers have *H pylori* infection.

Dynamics of H.pylori infection



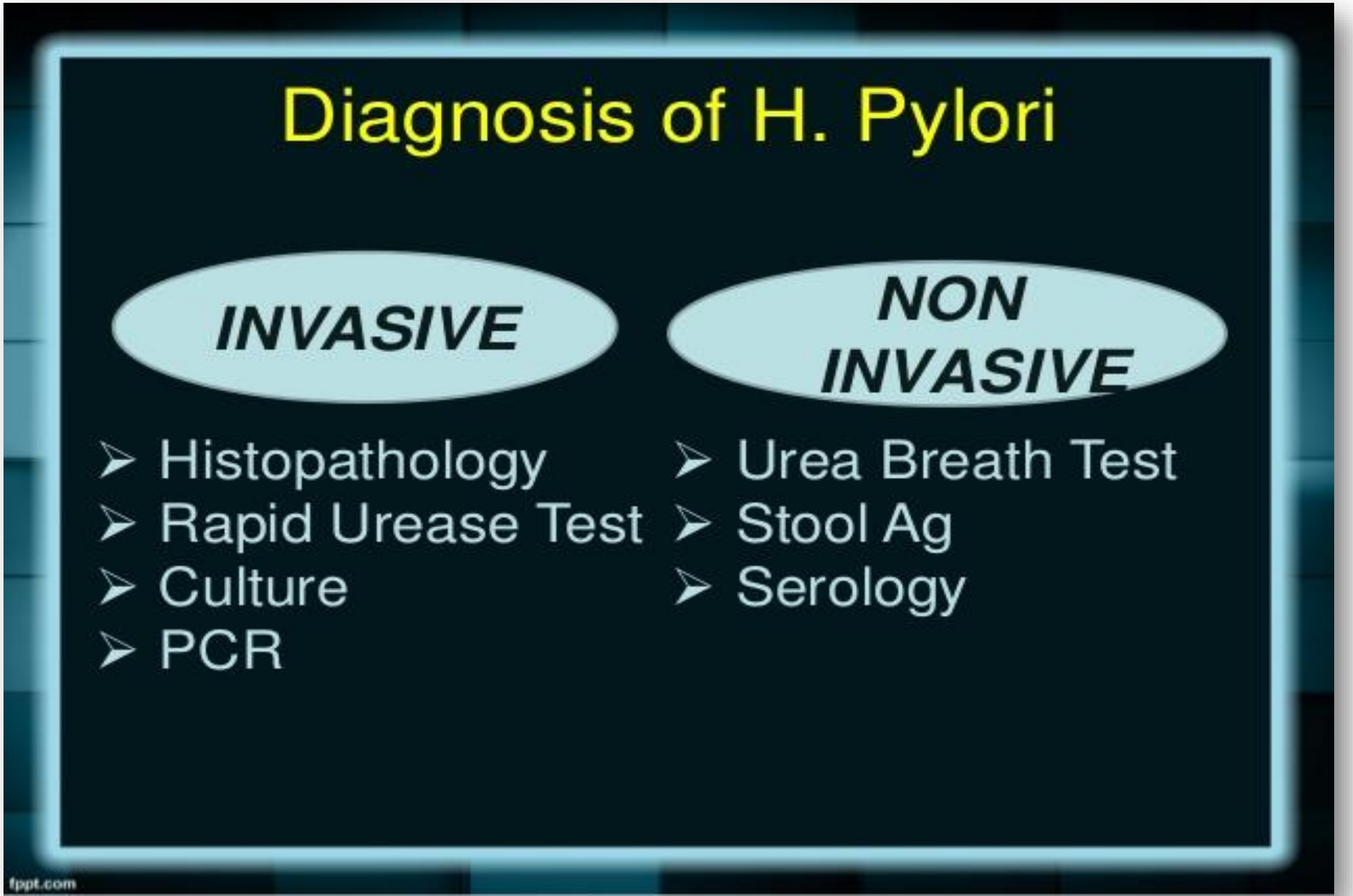
Symptoms of H.pylori infection

- Abdominal pain with burning or gnawing sensation.
- Pain is often made worse with empty stomach; night time pain is common.
- Poor appetite.
- Weight loss.
- Heart burn.
- Indigestion (dyspepsia)
- Belching.
- Nausea.
- Vomiting.
- Blood in stool.

Diagnostic Laboratory Tests

Specimens

Gastric biopsy
Blood
Stool samples



DIAGNOSIS BY NON INVASIVE METHODS

- Serology ELISA
- Urea breath test patient swallows urea solution

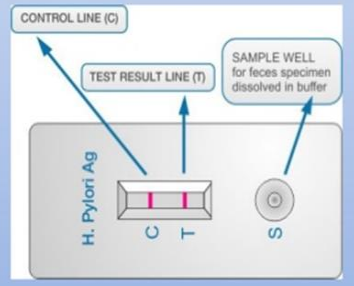
  In this test patient drinks urea solutions labeled with an isotope carbon

  If H.pylori is present in the urea is converted to ammonia and co2 in the breath measured.

Non invasive methods (No biopsy No endoscopy) for diagnosis of *H.pylori*

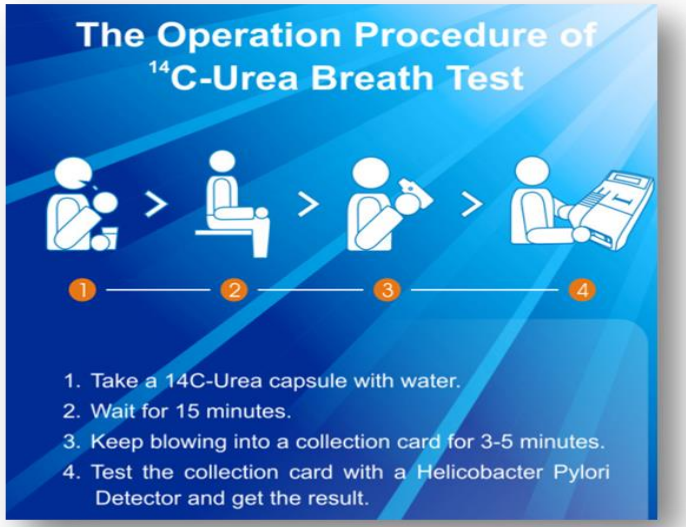
Diagnostic of H. Pylori Infection

3) **Stool antigen test.** A stool antigen test checks to see if substances that trigger the immune system to fight an *H. pylori* infection (*H. pylori* antigens) are present in your feces (stool). Stool antigen testing may be done to help support a diagnosis of *H. pylori* infection or to find out whether treatment for an *H. pylori* infection has been successful.

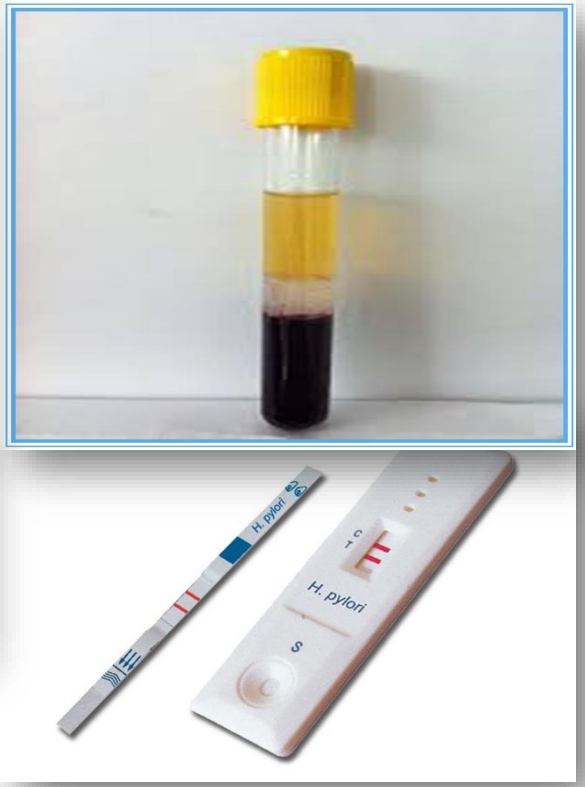


Stool antigen test

ELISA IgG,IgM,IgA

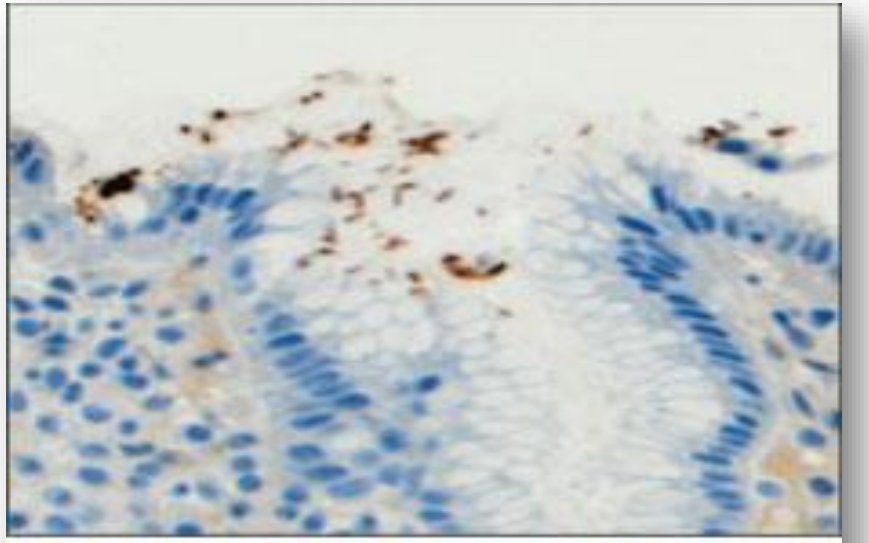


Urea breath test



Strip test for H.pylori
Screening test

Invasive methods (biopsy=endoscopy) for diagnosis of *H.pylori*

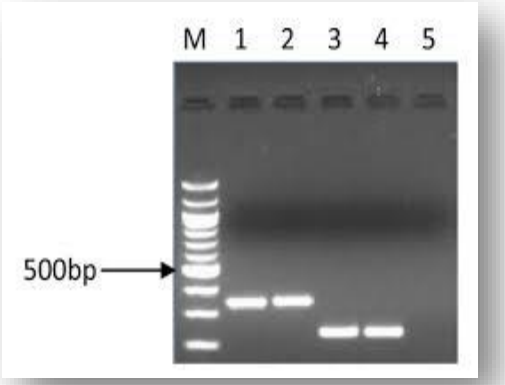


1. Immunohistochemical stain highlights the presence of Helicobacter pylori

Immunohistochemistry method

Rapid urease test

Culture



Molecular like PCR

Treatment
Triple therapy
Omeprazole 20 mg 1X2 For 14 days
Levofloxacin 100 1X1
Amoxicillin 500 1X3

But recurrent is common due to the drug resistant