

# Vibrios, Campylobacters, Helicobacter and Associated Bacteria

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# Overview

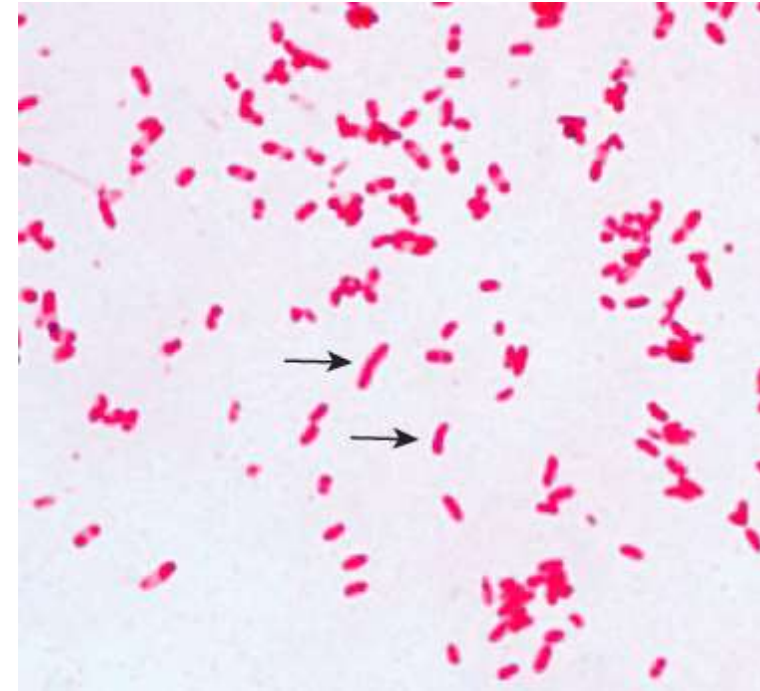
- These species are gram-negative rods that are all widely distributed in nature.
- *Vibrio cholerae* produces an enterotoxin that causes cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death.
- *Campylobacter jejuni* is a common cause of enteritis in humans.
- Less commonly, *Aeromonas* and, rarely, *Plesiomonas* have been associated with diarrheal disease in humans.
- *Helicobacter pylori* has been associated with gastritis and duodenal ulcer disease.

# THE VIBRIOS

- Vibrios are among the most common bacteria in surface waters worldwide.
- Vibrios cause a number of important infectious syndromes. Classic among them is cholera, a devastating diarrheal disease caused by *Vibrio cholerae* that has been responsible for seven global pandemics and much suffering over the past two centuries and remains a significant public health concern in the developing world today.
- *V. cholerae* serogroups **O1** and **O139** cause cholera in humans, and other vibrios may cause soft tissue infections, sepsis or enteritis.
- Other important *Vibrio* species associated primarily with gastrointestinal include *V. parahaemolyticus*, the most common cause of sea-foodborne (raw fish or shellfish) gastroenteritis in Asia, and *V. vulnificus* (oysters), a cause of severe sepsis in patients with cirrhosis and primary wound infection (*Vulnificus* is Latin for “wound maker.”) and *V. alginolyticus* occasionally causes eye, ear, and wound infections.

# VIBRIO CHOLERAE

- The epidemiology of cholera closely parallels the recognition of *V cholerae* transmission in water and the development of sanitary water systems.
- *V cholerae* is a comma-shaped, curved rod 2–4  $\mu\text{m}$  long . It is actively motile by means of a polar flagellum. On prolonged cultivation, vibrios may become straight rods that resemble the gram-negative enteric bacteria.



- Characteristically, vibrios grow at a very high pH (8.5–9.5) and are rapidly killed by acid.
- *V cholerae* produces convex, smooth, round colonies that are opaque and granular in transmitted light.
- *V cholera* 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.



- A positive oxidase test result is a key step in the preliminary identification of *V. cholerae* and other vibrios.
- *Vibrio* species are susceptible to the compound O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate), which differentiates them from *Aeromonas* species, which are resistant to O/129.
- Most *Vibrio* species are halotolerant, and NaCl often stimulates their growth. Some vibrios are halophilic, requiring the presence of NaCl to grow.

# Antigenic Structure and Biologic Classification

- ❖ Many vibrios share a single heat-labile flagellar **H** antigen. Antibodies to the H antigen are probably not involved in the protection of susceptible hosts.
- ❖ *V cholerae* has **O** lipopolysaccharides that confer serologic specificity. There are at least 206 O antigen groups.
  - *V cholerae* strains of O group 1 and O group 139 cause classic cholera; occasionally, non-O1/non-O139 *V cholerae* causes cholera-like disease.
  - Antibodies to the O antigens tend to protect laboratory animals against infections with *V cholerae*.
  - Two biotypes of *V. cholerae* O1, classical and El Tor, are distinguished. Each biotype is further subdivided into two serotypes, termed Inaba and Ogawa.

# Vibrio cholerae Enterotoxin

- Cholera toxin, a potent protein enterotoxin elaborated by the organism in the small intestine with a molecular weight (MW) of about 84,000, consisting of subunits A (MW,28,000) and B.
- The genes for V cholerae enterotoxin are on the bacterial **chromosome**.
- 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.



# Pathogenesis

- Under natural conditions, *V cholerae* is pathogenic only for humans. A 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.
- The toxin-coregulated pilus (TCP), so named because its synthesis is regulated in parallel with that of cholera toxin, is essential for *V. cholerae* to survive and multiply in (colonize) the small intestine.
- The organisms do not reach the bloodstream but remain within the intestinal tract.
- Virulent *V cholerae* organisms attach to the microvilli of the brush border of epithelial cells. There they multiply and liberate cholera toxin and perhaps mucinases and endotoxin.

# Clinical Findings

- The burden of disease is often greatest during “cholera seasons” associated with high temperatures, heavy rainfall, and flooding, but cholera can occur year-round.
- About 50% of infections with classic V cholerae are asymptomatic, as are about 75% of infections with the El Tor biotype.
- The incubation period is 12 hours–3 days for persons who develop symptoms, depending largely on the size of the inoculum ingested.
- 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. The mortality rate without treatment is between 25% and 50%.
- The diagnosis of a full blown case of cholera presents no problem in the presence of an epidemic. However, sporadic or mild cases are not readily differentiated from other diarrheal diseases. The El Tor biotype tends to cause milder disease than the classic biotype.



# Diagnostic Laboratory Tests

- A. Specimens
- Specimens for culture consist of mucus flecks from stools.
  
- B. Smears
- Dark-field or phase contrast microscopy may show the rapidly motile vibrios.
  
- C. Culture
- Growth is rapid in peptone agar, on blood agar with a pH near 9.0, or on TCBS agar, and typical colonies can be picked in 18 hours.
  
- D. Specific Tests
- *V cholerae* organisms are further identified by slide agglutination tests using anti-O group 1 or group 139 antisera and by biochemical reaction patterns.

# Treatment

- The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion.
- Many antimicrobial agents are effective against *V cholerae*, but these play a secondary role in patient management. Oral tetracycline and doxycycline tend to reduce stool output in cholera and shorten the period of excretion of vibrios.
- In some endemic areas, tetracycline resistance of *V cholerae* has emerged; the genes are carried by transmissible plasmids. In children and pregnant women, alternatives to the tetracyclines include erythromycin and furazolidine .

# Prevention

- ❖ Provision of safe water and of facilities for sanitary disposal of feces, improved nutrition, and attention to food preparation and storage in the household can significantly reduce the incidence of cholera.
- ❖ Currently, two oral killed cholera vaccines have been prequalified by the WHO and are available internationally :
  - WC-rBS (Dukoral.; Crucell, Stockholm, Sweden) contains several biotypes and serotypes of *V. cholerae* O1 supplemented with recombinant cholera toxin B subunit.
  - BivWC (Shanchol™; Shantha Biotechnics–Sanofi Pasteur, Mumbai, India) contains several biotypes and serotypes of *V. cholerae* O1 and *V. cholerae* O139 without supplemental cholera toxin B subunit.

# CAMPYLOBACTER

- Campylobacters are motile, non-spore-forming, curved, gram-negative rods.
- Campylobacters are found in the gastrointestinal tract of many animals used for food (including poultry, cattle, sheep, and swine) and many household pets (including birds, dogs, and cats)
- Campylobacters cause both diarrheal and systemic diseases and are among the most widespread causes of infection in the world.
- The classification of bacteria within the family Campylobacteriaceae has changed frequently. Some species previously classified as campylobacters have been reclassified in the genus *Helicobacter*. The genus *Arcobacter* has been created.
- The human pathogens fall into two major groups: those that primarily cause diarrheal disease and those that cause extraintestinal infection

- *Campylobacter jejuni* is the prototype organism in the group and is a very common cause of diarrhea in humans.
- *Campylobacter fetus* has two subspecies, *fetus* and *venerealis*. *C fetus* subspecies *fetus* is an opportunistic pathogen that causes systemic infections in immunocompromised patients. It may occasionally cause diarrhea
- Other organisms that cause diarrheal disease include *Campylobacter coli*, *Campylobacter upsaliensis*, *Campylobacter lari*, *Campylobacter hyointestinalis*, *Campylobacter fetus*, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Helicobacter cinaedi*, and *Helicobacter fennelliae*.

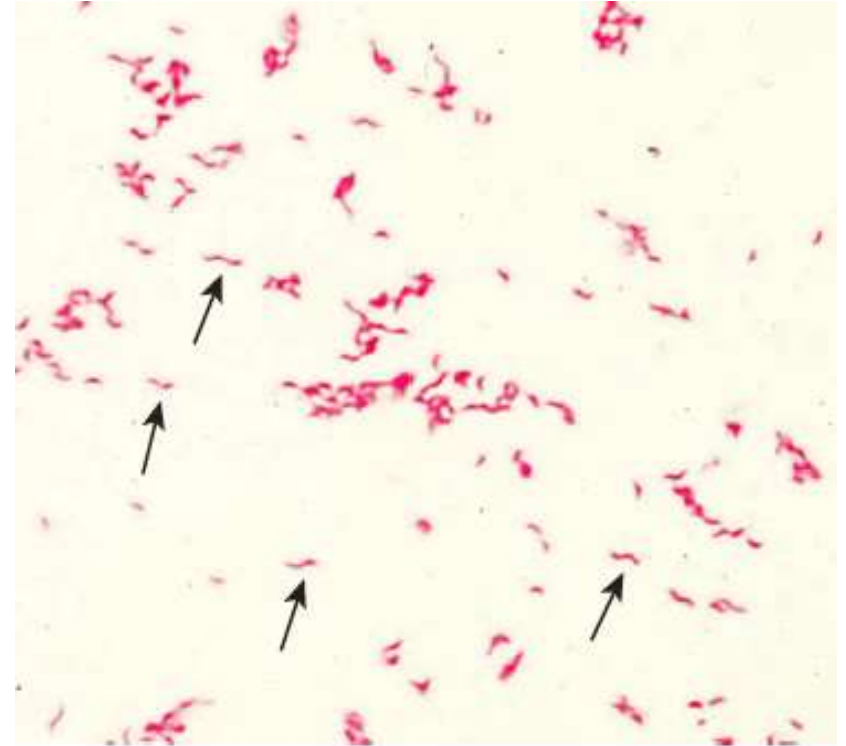


# CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI

- C jejuni and Campylobacter coli have emerged as common human pathogens, causing mainly enteritis and occasionally systemic infection.
- C jejuni and C coli cause infections that are clinically indistinguishable, and laboratories generally do not differentiate between the two species.
- Between 5% and 10% of infections reported to be caused by C jejuni are probably caused by C coli. These bacteria are at least as common as salmonellae and shigellae as a cause of diarrhea especially in the developed world.

# CAMPYLOBACTER JEJUNI

- gram-negative rods with comma, S, or “**gull wing**” shapes. They are motile, with a single polar flagellum, and do not form spores.
- Selective media are needed, and incubation must be in an atmosphere with reduced O<sub>2</sub> (5% O<sub>2</sub>) with added CO<sub>2</sub> (10% CO<sub>2</sub>).
- Incubation of primary plates for isolation of C jejuni should be at 42°C. Although C jejuni grows well at 36–37°C, incubation at 42°C prevents growth of most of the other bacteria present in feces, thus simplifying the identification of C jejuni. Several selective media are in widespread use.



# Pathogenesis

- The infection is acquired by the oral route from food, drink, or contact with infected animals or animal products, especially poultry.
- *C jejuni* is susceptible to gastric acid, and ingestion of about  $10^4$  organisms is usually necessary to produce infection.
- Both the motility of the strain and its capacity to adhere to host tissues appear to favor disease, but classic enterotoxins and cytotoxins (cytolethal distending toxin, or CDT) appear not to play substantial roles in tissue injury or disease production.
- The organisms multiply in the small intestine, invade the epithelium, and produce inflammation that results in the appearance of red and white blood cells in the stools. Occasionally, the bloodstream is invaded, and a clinical picture similar to enteric fever develops. Localized tissue invasion coupled with the toxic activity appears to be responsible for the enteritis.

# Clinical Findings

- A prodrome of fever, headache, myalgia, and/or malaise often occurs 12–48 h before the onset of diarrheal symptoms. profuse diarrhea that may be grossly bloody.
- Usually the illness is self-limited to a period of 5–8 days, but occasionally it continues longer.
- Most cases resolve without antimicrobial therapy; however, in about 5–10% of patients, symptoms may recur.
- Local suppurative complications of infection include cholecystitis, pancreatitis, and cystitis; distant complications include meningitis, endocarditis, arthritis, peritonitis, cellulitis, and septic abortion. All these complications are rare, except in immunocompromised hosts
- Hepatitis, interstitial nephritis, and the hemolytic-uremic syndrome occasionally complicate acute infection
- Certain serotypes of *C jejuni* have been associated with post-diarrheal Guillain-Barré syndrome, a form of ascending paralytic disease. Reactive arthritis and Reiter's syndrome may also follow acute campylobacter diarrhea.

# Diagnostic Laboratory Tests

- A. Specimens
  - Diarrheal stool is the usual specimen. *C jejuni* , *C fetus* , and other campylobacters may occasionally be recovered from blood cultures usually from immunocompromised or elderly patients.
- B. Smears
  - Gram-stained smears of stool may show the typical “**gull wing**”–shaped rods. Dark-field or phase contrast microscopy may show the typical **darting motility** of the organisms.
- C. Culture
  - Culture on the selective media (Skirrow's, Butzler's, Blaser's, Campy-BAP and Preston media ) is the definitive test to diagnose *C jejuni* enteritis. If another species of *Campylobacter* is suspected, medium without a cephalosporin should be used and incubated at 36–37° C.

# Treatment

- Fluid and electrolyte replacement is central to the treatment of diarrheal illnesses.
- Even among patients presenting for medical attention with *Campylobacter* enteritis, not all clearly benefit from specific antimicrobial therapy. Indications for therapy include high fever, bloody diarrhea, severe diarrhea, persistence for >1 week, and worsening of symptoms. A 5- to 7-day course of erythromycin is the regimen of choice.
- An alternative regimen for adults is ciprofloxacin or another fluoroquinolone for 5–7 days.
- For systemic infections, treatment with gentamicin or imipenem or chloramphenicol should be started empirically, but susceptibility testing should then be performed

# HELICOBACTER PYLORI

- H pylori is a **spiral-shaped** gram-negative rod.
- It has **multiple flagella** at one pole and is actively motile.
- The organism has several acid-resistance mechanisms, most notably a highly expressed **urease** that catalyzes urea hydrolysis to produce buffering ammonia. H. pylori is microaerophilic (i.e., requires low levels of oxygen), is oxidase positive and catalase positive is slow-growing, and requires complex growth media in vitro.
- H pylori is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphomas. It may be one initial precipitant of pernicious anemia and also may predispose some patients to iron deficiency through occult blood loss and/or hypochlorhydria and reduced iron absorption.

# Epidemiology

- *Helicobacter pylori* colonizes the stomach in ~50% of the world's human population, essentially for life unless eradicated by antibiotic treatment.
- Humans are the only important reservoir of *H. pylori*. Children may acquire the organism from their parents (most often the primary caregiver) or from other children.
- Most *H. pylori*-colonized persons do not develop clinical sequelae. That some persons develop overt disease whereas others do not is related to a combination of factors: bacterial strain differences (cag-positive, type IV secretion system, the vacuolating cytotoxin VacA), host susceptibility to disease, and environmental factors (the interleukin 1 gene polymorphisms, and smoking).



# Pathogenesis

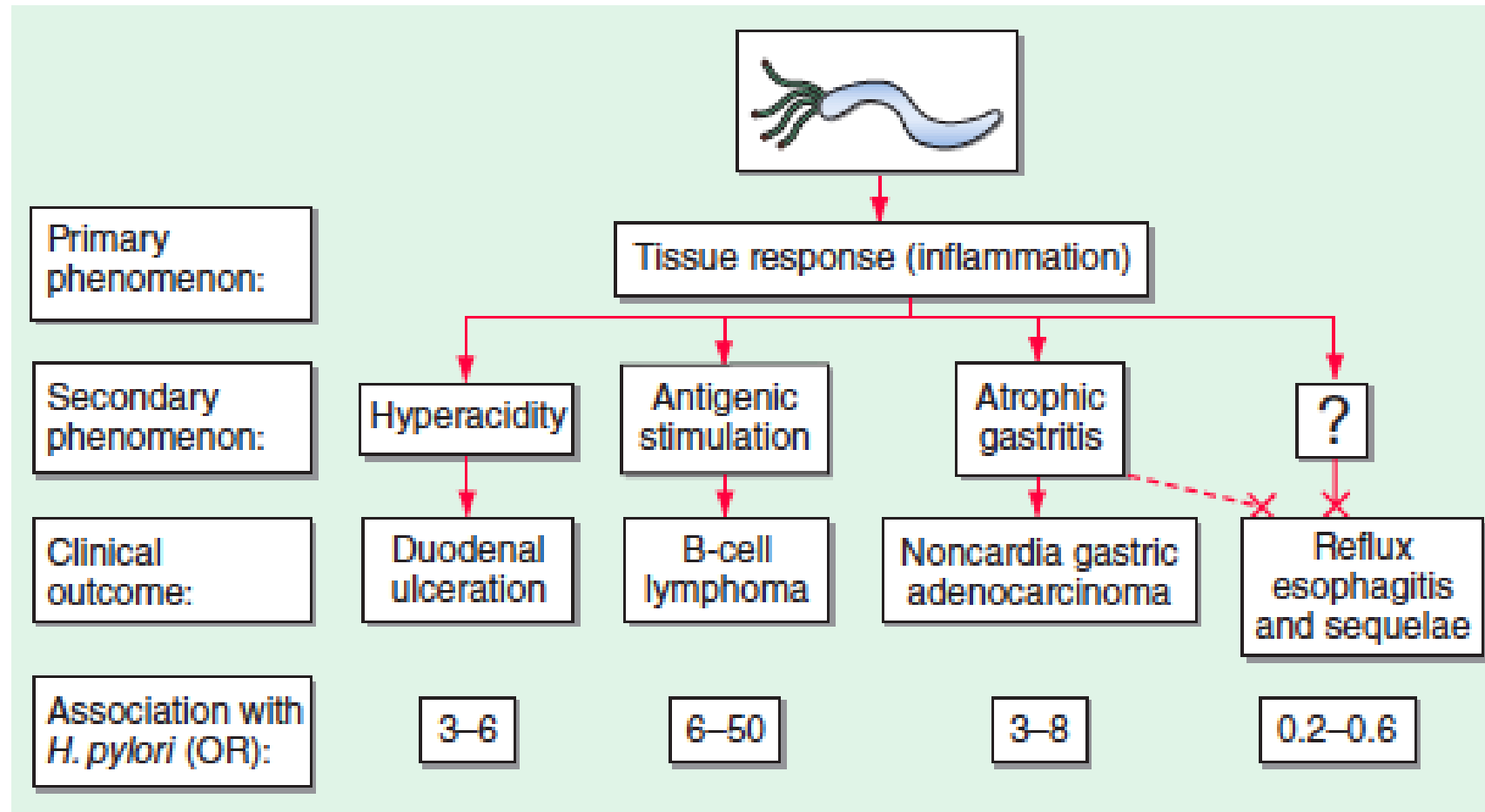
- H pylori is found deep in the mucous layer near the epithelial surface where physiologic pH is present.
- H pylori is quite motile, even in mucus, and is able to find its way to the epithelial surface. H pylori overlies gastric-type but not intestinal- type epithelial cells.
- H pylori also produces 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.

- The mechanisms by which H pylori causes mucosal inflammation and damage are not well defined but probably involve both bacterial and host factors. The bacteria invade the epithelial cell surface to a limited degree. Toxins and lipopolysaccharide may damage the mucosal cells, and the ammonia produced by the urease activity may also directly damage the cells.
- Polymorphonuclear and mononuclear cell infiltrates are seen within the epithelium and lamina propria. Vacuoles within cells are often pronounced. Destruction of the epithelium is common, and glandular atrophy may occur. H pylori thus is a major risk factor for gastric cancer.
- H. pylori colonization induces chronic superficial gastritis, a tissue response in the stomach that includes infiltration of the mucosa by both mononuclear and polymorphonuclear cells.

# Clinical Findings

- Acute infection can yield an upper gastrointestinal illness with nausea and pain; vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks.
- After colonization, the H pylori infection persists for years and perhaps decades or even a lifetime. About 90% of patients with duodenal ulcers and 50–80% of those with gastric ulcers have H pylori infection. Recent studies confirm that H pylori also is a risk factor for gastric carcinoma and lymphoma.

# Relationships between colonization with *Helicobacter pylori* and diseases of the upper gastrointestinal tract.



# Diagnostic Laboratory Tests

## ❖ Smears

- The diagnosis of gastritis and H pylori infection can be made histologically. A gastroscopy procedure with biopsy is required. Routine stains demonstrate gastritis, and Giemsa or special silver stains can show the curved or spiral-shaped organisms.

## ❖ Culture

- .Culture is performed when patients are not responding to treatment, and there is a need to assess susceptibility patterns.

## ❖ Special Tests

- Rapid tests to detect urease activity in vitro are widely used for presumptive identification of H pylori in specimens.
- In vivo tests for urease activity can be done also. In urea breath tests,  $^{13}\text{C}$ - or  $^{14}\text{C}$ -labeled urea is ingested by the patient. If H pylori is present, the urease activity generates labeled  $\text{CO}_2$  that can be detected in the patient's exhaled breath.
- Detection of H pylori antigen in stool specimens is appropriate as a test of cure for patients with known H pylori infection who have been treated.

# Treatment

- Triple therapy with metronidazole and either bismuth subsalicylate or bismuth subcitrate plus either amoxicillin or tetracycline for 14 days eradicates H pylori infection in 70–95% of patients.
- An acid-suppressing agent given for 4 or 6 weeks enhances ulcer healing. Proton pump inhibitors (PPIs) directly inhibit H pylori and appear to be potent urease inhibitors.
- The preferred initial therapy is 7–10 days of a PPI plus amoxicillin and clarithromycin or a quadruple regimen of a PPI metronidazole, tetracycline, and bismuth for 10 days.

# PLESIOMONAS

- Un-commonly, Plesiomonas have been associated with diarrheal disease in humans.
- Plesiomonas shigelloides is an oxidase positive, gram-negative rod with polar flagella.
- Plesiomonas is most common in tropical and subtropical areas. It is a water and soil organism and has been isolated from freshwater fish and many animals.
- Most isolates from humans have been from stool cultures of patients with diarrhea.
- Plesiomonas grows on the differential media used to isolate Salmonella and Shigella from stool specimens .
- Some Plesiomonas strains share antigens with Shigella sonnei, and cross-reactions with Shigella antisera occur. Plesiomonas can be distinguished from shigellae in diarrheal stools by the oxidase test: Plesiomonas is oxidase positive, and shigellae are not.
- Plesiomonas species are positive for DNase; this and other biochemical tests distinguish it from Aeromonas species.

# Aeromonas

- Aeromonas species are distinguished from the enteric gram-negative rods by finding a positive oxidase reaction in growth obtained from a blood agar plate. Aeromonas species are differentiated from vibrios by showing resistance to the compound O/129 and lack of growth on media containing 6% NaCl.
- Typically, aeromonads produce hemolysins. Some strains produce an enterotoxin, Cytotoxins and the ability to invade cells in tissue culture have been noted.
- However, gastroenteritis, caused mostly by *A. caviae* complex, ranges from acute watery diarrhea to less commonly a dysenteric type of illness.
- Aeromonas species are also associated with extraintestinal infections such as bacteremia and wound infections. The latter are often the result of trauma that occurs in a water environment and are caused primarily by *A. hydrophila*



# Treatment

- Aeromonas and Plesiomonas species are generally susceptible to fluoroquinolones (e.g., ciprofloxacin), third- and fourth-generation cephalosporins carbapenems, and aminoglycosides, but resistance has been described to all those agents. Because Aeromonas can produce various  $\beta$ -lactamases, including carbapenemases,
- Susceptibility testing must be used to guide therapy.

The End







