Gram-negative rods widely distributed in nature

	VIBRIO	AEROMONAS	CAMPYLOBACTER	HELICOBACTER
Habitat / Distribution	Marine and surface waters 	Fresh and brackish waters 	Animals (domesticated animals) 	Gastrointestinal and hepatobiliary tracts of humans and various other mammals  • Humans • Dogs • Cats • Cattle • Dolphins • Chickens • Wild birds
Important Diseases	**Cholera** Profuse watery diarrhea due to enterotoxin; can rapidly lead to dehydration and death. 	Gastroenteritis, skin and soft tissue infections, sepsis 	**Enteritis** (inflammatory diarrhea) 	Gastritis and duodenal ulcer disease 
Example Species	• *Vibrio cholerae* • *Vibrio parahaemolyticus* • *Vibrio vulnificus*	• *Aeromonas hydrophila* • *Aeromonas caviae*	• Campylobacter jejuni	• Helicobacter pylori

THE VIBRIOS

- Comma-shaped, facultatively anaerobic rods; catalase and oxidase positive, and most species are motile by means of polar flagella.
- Grow within a broad temperature range (14–40°C), high pH range (8.5–9.5), require sodium chloride (NaCl) for growth – halophilic ("salt loving").
- *V. cholerae* serogroups O1 and O139 cause cholera in humans, *V. parahaemolyticus* and *V. vulnificus* can cause skin and soft tissue infections, sepsis, or gastroenteritis.
- Cholera is associated with poor sanitation, direct contact with or consumption of contaminated water and/or food.

Vibrio cholerae (SEM)

VIBRIO CHOLERAE – MORPHOLOGY & IDENTIFICATION

- *V. cholerae* is a comma-shaped, curved motile rod, grow well at 37°C on routine media as well as selective media such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar.
- *V. cholerae* produces yellow colonies (sucrose fermented) on TCBS agar, while *V. parahaemolyticus* and *V. vulnificus* (non sucrose fermenetrs) produce green colonies.
- Stool specimens should be collected early in the course of the diarrheal illness.
- In endemic areas, stool cultures on TCBS and enrichment broth culture are appropriate.

TCBS AGAR COLONIES

V. cholerae (sucrose fermenter)	*V. parahaemolyticus* (non-sucrose fermenter)	*V. vulnificus* (non-sucrose fermenter)
Yellow colonies	Green colonies	Green colonies

STOOL COLLECTION

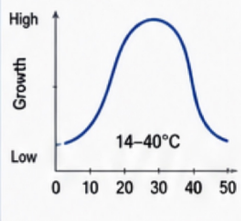
Collect early in the course of diarrheal illness.

ENRICHMENT BROTH CULTURE

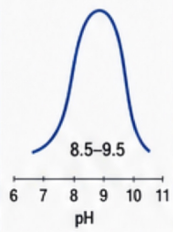
Alkaline peptone water (APW) enrichment followed by subculture on TCBS agar.

GROWTH CHARACTERISTICS OF VIBRIOS

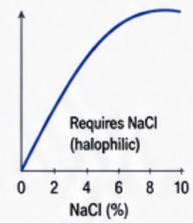
TEMPERATURE RANGE (°C)



pH RANGE



NaCl REQUIREMENT (%)



POLAR FLAGELLUM (MOTILITY)

BIOCHEMICAL & ANTIGENIC PROPERTIES OF *V. CHOLERAE*

CARBOHYDRATE FERMENTATION

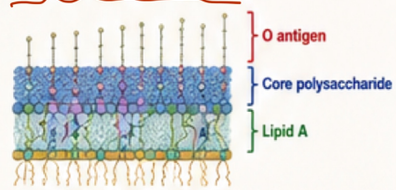
- ✓ Sucrose (fermented)
- ✓ Mannose (fermented)
- ✗ Arabinose (not fermented)

OTHER PROPERTIES

- Oxidase positive
- Can grow on most agar media without additional salt

O ANTIGEN (LIPOPOLYSACCHARIDE)

- *V. cholerae* has O lipopolysaccharides antigen (200+ serogroups).
- Serogroup O1 and O139 cause epidemic and pandemic cholera.
- Non-O1/non-O139 strains can cause cholera-like diarrheal disease.



Lipopolysaccharide (LPS)

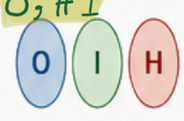
V. CHOLERAE O1 SEROTYPES & BIOTYPES

O1 SEROTYPES

Three serotypes:

1. Ogawa
2. Inaba
3. Hikojima

O, HI



BIOTYPES OF EPIDEMIC *V. CHOLERAE* O1

Two biotypes: الطَّوْر

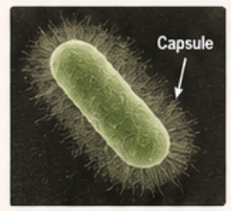
CLASSIC	EL TOR
• Older pandemic strain	• Current pandemic strain
• No hemolysin	• Produces hemolysin
• Sensitive to polymyxin B	• Resistant to polymyxin B

HEMOLYSIS ON BLOOD AGAR (EL TOR)

CAPSULE PRODUCTION

PRODUCE ACIDIC POLYSACCHARIDE CAPSULE

- O139 strains
- Non-O1 strains
- *Vibrio vulnificus*



(Electron micrograph)

DO NOT PRODUCE CAPSULE

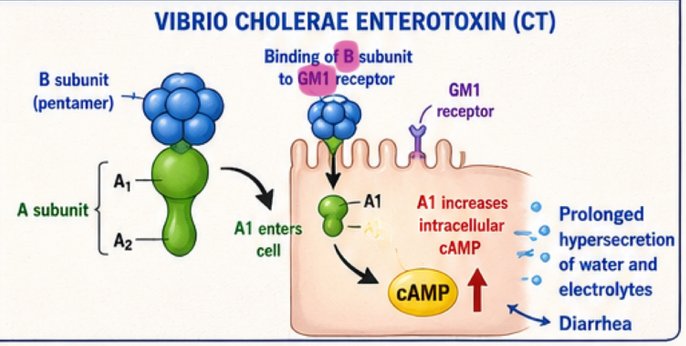
- *V. cholerae* O1 strains

(Electron micrograph)

IMPORTANCE OF CAPSULE → ① (Resist) phagocytosis ② enhance survival in nature ③ ↑ virulence

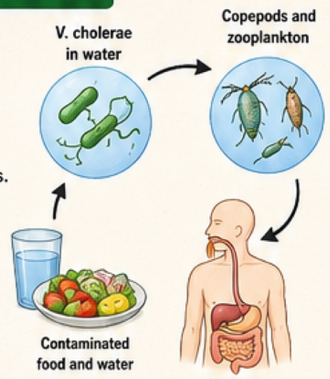
VIBRIO CHOLERAE ENTEROTOXIN

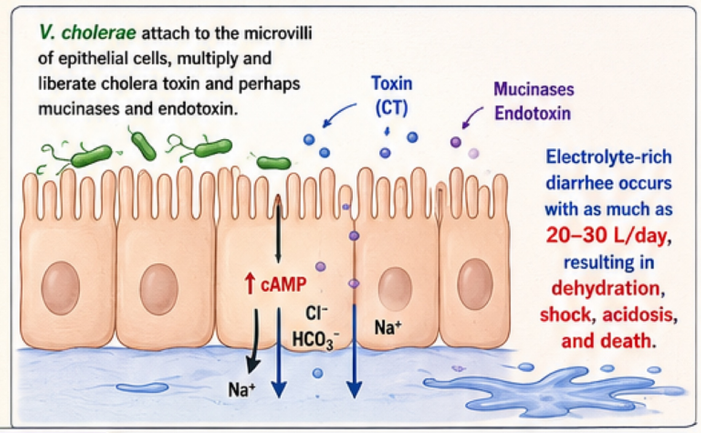
- Is a heat-labile enterotoxin consist of A and B subunits, subunit A1 increases intracellular cAMP: prolonged hypersecretion of water and electrolytes.
- *V. cholerae* organisms attach to the microvilli of epithelial cells, multiply and liberate cholera toxin and perhaps mucinases and endotoxin.
- Electrolyte-rich diarrhea occurs with as much as 20–30 L/day, resulting in dehydration, shock, acidosis, and death.



PATHOGENESIS AND PATHOLOGY

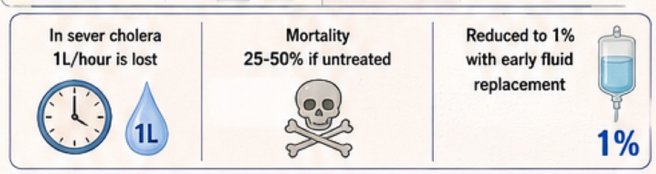
- *V. cholerae* is pathogenic only for humans and grow in association with copepods and zooplankton.
- Contaminated food and water are more likely source of infections.
- Persons with achlorhydria or hypochlorhydria (or on PPI) require lower infectious dose (10^3 vs 10^{10})

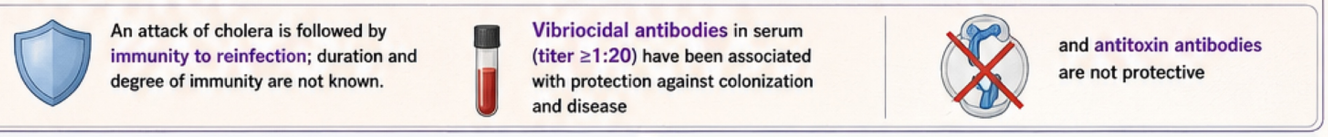




CLINICAL FINDINGS

- Disease severity ranges from asymptomatic intestinal colonization to severe diarrhea.
- The incubation period is 12 hours to 3 days for persons who develop symptoms, depending on the size of the inoculum ingested.
- A sudden onset of nausea and vomiting, followed by profuse diarrhea with abdominal cramps; "rice water stool".
- In sever cholera 1L/hour is lost; dehydration can lead to shock and collapse; mortality 25–50% if untreated, reduced to 1% with early fluid replacement.
- The *V. cholerae* O1 El Tor biotype tends to cause milder disease than the classic biotype.
- An attack of cholera is followed by immunity to reinfection; duration and degree of immunity are not known.
- Vibriocidal antibodies in serum (titer ≥1:20) have been associated with protection against colonization and disease and antitoxin antibodies are not protective.



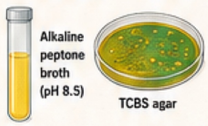


DIAGNOSTIC LABORATORY TESTS

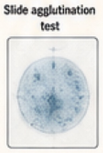
- Specimens: stool specimens collected early in the course of the diarrheal illness, inoculated within 2–4 hours of collection, mixed in a Cary-Blair if delayed.

- On Dark-field or phase-contrast microscopy; "shooting star" motility after mixing with a polyvalent O 1 antisera is suggestive of *V. cholerae* O1.

- Culture: grow well on most agar media, growth is rapid in alkaline peptone broth or water, containing 1% NaCl with a pH of 8.5, or on TCBS agar.



- *V. cholerae* organisms are further identified by slide agglutination tests using anti-O group 1 or group 139 antisera and biochemical reaction patterns.



Biochemical reaction patterns

Oxidase	+
Indole	−
TSI	A/A, no H_2S
Citrate	+

TREATMENT

- Water and electrolyte replacement to correct the severe dehydration and salt depletion is the most important.

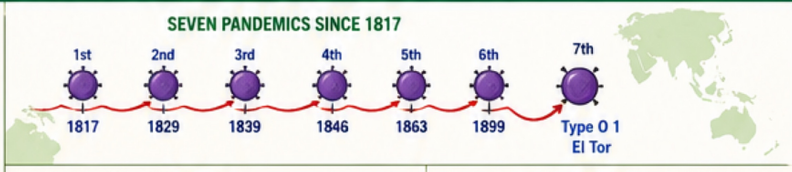
- Appropriate antimicrobial therapy can also reduce the duration and amount of shedding of *Vibrio* organisms in the stool.

- Tetracycline, trimethoprim–sulfamethoxazole, fluoroquinolones, and doxycycline are effective; erythromycin and/or azithromycin are an appropriate choice of antimicrobial therapy in children and in pregnant women

EPIDEMIOLOGY, PREVENTION, AND CONTROL

- Seven pandemics since 1817 have been recorded caused by classic O 1 biotype, except the seventh is type O 1 El Tor.
- Cholera is spread by contact involving individuals with mild or early illness and by water, food, and flies; 1–5% of exposed susceptible persons develop disease.
- Infected people shed only during the first few days of the illness; no long-term carriage in humans; seldom exceeds 3–4 weeks.
- Education and improved sanitation, isolation of patients and disinfection are important preventive measures.
- Antimicrobial treatment reduce clinical symptoms and transmission; chemoprophylaxis to household contacts limit spread of infection.
- Cholera vaccines are available and are advised when traveling to endemic area

SEVEN PANDEMICS SINCE 1817



SPREAD

Contact involving individuals with mild or early illness

By water, food,

and flies

1–5% of exposed susceptible persons develop disease.

SHEDDING

Infected people shed only during the first few days of the illness; no long-term carriage in humans; seldom exceeds 3–4 weeks.

PREVENTIVE MEASURES

Education | Improved sanitation | Isolation of patients | Disinfection

CHEMOPROPHYLAXIS & VACCINATION

Antimicrobial treatment reduce clinical symptoms and transmission.

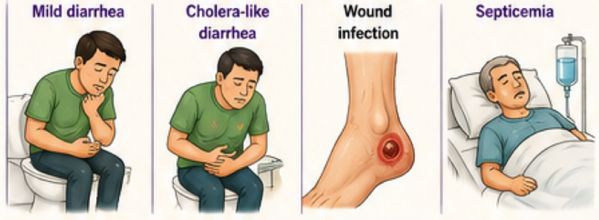
Chemoprophylaxis to household contacts limit spread of infection.

Cholera vaccines are available and are advised when traveling to endemic area

NON-O1 AND NON-O139 *V. CHOLERAE*

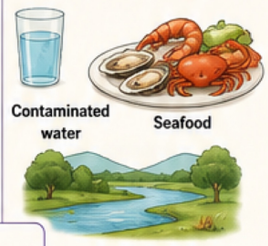
- Cholera-like diarrhea and extraintestinal disease
- *V. cholerae* strains outside O1 and O139 are generally not associated with classic epidemic cholera.
- These strains may cause mild diarrhea, cholera-like diarrhea, wound infection, or septicemia.
- Disease is usually associated with contaminated water, seafood, or environmental exposure.
- Some strains may produce toxins or other virulence factors, but they usually lack the classic epidemic combination of O1/O139 serogroup and cholera toxin.
- These infections are important in patients with comorbidities or after exposure to contaminated aquatic environment

CLINICAL MANIFESTATIONS



Mild diarrhea | Cholera-like diarrhea | Wound infection | Septicemia

ASSOCIATED WITH



Contaminated water | Seafood | Environmental exposure

Toxins / Virulence Factors

Some strains may produce toxins or other virulence factors (usually lack the classic epidemic combination of O1/O139 serogroup and cholera toxin).

Important in

Patients with comorbidities | After exposure to contaminated aquatic environment

VIBRIO PARAHAEMOLYTICUS

- *V. parahaemolyticus* is a halophilic *Vibrio* species associated with marine environments.
- Infection commonly follows ingestion of raw or undercooked seafood, especially shellfish.
- The illness is usually acute gastroenteritis with watery diarrhea, abdominal cramps, nausea, vomiting, and sometimes fever.
- Some cases may have dysentery-like features.
- Diagnosis is by stool culture using appropriate selective methods, and the illness is usually self-limited

SOURCE

Raw or undercooked seafood, especially shellfish

CLINICAL FEATURES

Watery diarrhea | Abdominal cramps | Nausea, vomiting | Fever | Some cases may have dysentery-like features

DIAGNOSIS

Stool culture using appropriate selective methods

Illness is usually self-limited

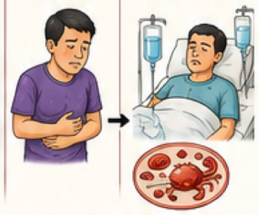
VIBRIO VULNIFICUS

- *V. vulnificus* is an important halophilic *Vibrio* species associated with seawater and shellfish, especially oysters.
- Infection may occur after ingestion of contaminated raw oysters or after wound exposure to seawater.
- Gastrointestinal symptoms may be followed by rapidly progressive septicemia.
- Wound infection may progress to cellulitis, bullae, necrotizing fasciitis, and septic shock.
- Severe disease is more likely in patients with liver disease, alcoholism, hemochromatosis, immunosuppression, or chronic illness

ROUTE OF INFECTION

Ingestion of contaminated raw oysters | Wound exposure to seawater

CLINICAL MANIFESTATIONS



Gastrointestinal symptoms | Rapidly progressive septicemia

WOUND INFECTION MAY PROGRESS TO

Cellulitis | Bullae | Necrotizing fasciitis | Septic shock

SEVERE DISEASE IS MORE LIKELY IN

Liver disease | Alcoholism | Hemochromatosis | Immunosuppression | Chronic illness

AEROMONAS

- *Aeromonas* are ubiquitous inhabitants of fresh and brackish water.
- They are Gram-negative, facultative anaerobic rods that ferment carbohydrates and may resemble Enterobacteriaceae, but *Aeromonas* are oxidase-positive.
- They grow on blood agar and enteric differential/selective agars; on blood agar they are usually β-hemolytic.
- Main human-associated species: *A. hydrophila*, *A. caviae*, and *A. veronii* biovar sobria.
- *A. caviae* is most frequently asconiated with gastroenteritis, ranging from watery diarrhea to dysentery-like illness, with abdominal pain, fever, nausea, and vomiting.
- Infection follows ingestion of contaminated food or water, especially during warm summer months.
- Gastroenteritis is usually self-limited; severe diarrhea, dehydration, or infection in children may require hospitalization

HABITAT

MORPHOLOGY & BIOCHEMISTRY

- Gram-negative
- Facultative anaerobic rods
- Ferment carbohydrates
- May resemble Enterobacteriaceae
- Oxidase-positive

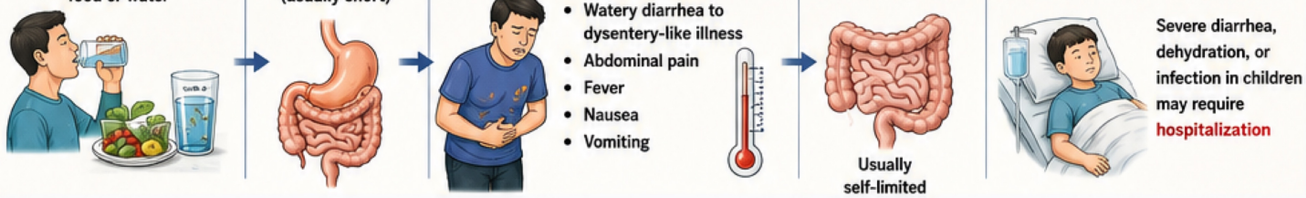
Oxidase +

CULTURE CHARACTERISTICS

MAIN HUMAN-ASSOCIATED SPECIES

A. hydrophila | *A. caviae* | *A. veronii* biovar sobria

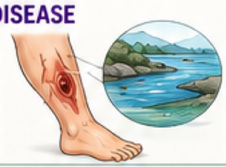
A. CAVIAE–ASSOCIATED GASTROENTERITIS



EXTRAINTESTINAL DISEASE, DIAGNOSIS, AND TREATMENT

EXTRAINTESTINAL DISEASE

Wound Infections (*A. hydrophila*)



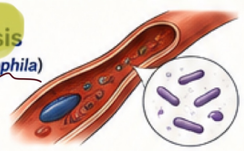
- *A. hydrophila* causes wound infections, usually after traumatic injury exposed to fresh or brackish water.
- Most commonly, cellulitis develops within 48 hours, but fasciitis, myonecrosis, osteomyelitis, and systemic symptoms may occur.

Medicinal Leeches

- Aeromonas soft tissue infection may rarely follow use of medicinal leeches.

وهو نوع من الديدان يُستخدم طبيًا لسحب الدم وتحسين التروية الدموية في بعض الجراحات

Aeromonas Sepsis (mostly due to *A. hydrophila*)

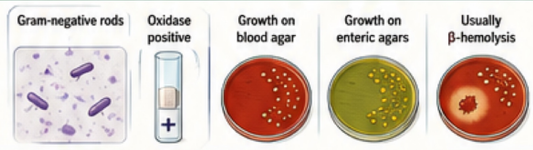


- Aeromonas sepsis, mostly due to *A. hydrophila*, occurs mainly in patients with hematologic malignancies and/or liver disease.

Risk Factors

DIAGNOSIS

- Diagnosis is suggested by Gram-negative rods, oxidase positivity on blood agar/enteric agars, and usually β-hemolysis.



GASTROENTERITIS TREATMENT

- Gastroenteritis usually needs no antimicrobial therapy, but severe cases may improve faster with treatment.

Supportive care (fluids, rest)

Antimicrobial therapy for severe cases

ANTIMICROBIAL RESISTANCE

- Clinically significant aeromonads are resistant to penicillin and ampicillin, often resistant to cefazolin and ticarcillin.

ACTIVE OPTIONS

- Active options include third-generation cephalosporins, aztreonam, carbapenems, aminoglycosides, and especially fluoroquinolones; empiric therapy may use two or more agents until susceptibility results are available.

Empiric therapy: may use two or more agents until susceptibility results are available.

CAMPYLOBACTER

- Campylobacters cause both diarrheal and systemic diseases and are among the most widespread causes of infection worldwide.
- *C. jejuni* is the prototype organism and is a very common cause of diarrhea in humans; other campylobacters include *C. fetus*, *C. coli*, and *C. upsaliensis*.
- *C. jejuni* and other campylobacters are curved, comma-, or S-shaped, Gram-negative, non-spore-forming rods and may have "sea gull wing" shapes.
- Campylobacters are motile, with a single polar flagellum at one or both ends, although some organisms may lack flagella.
- Campylobacter species multiply more slowly than other Gram-negative enteric bacteria, so selective media containing antibiotics are needed for stool isolation.
- Examples of selective media include Campy-Blood agar and Skirrow's medium.

camping takes time

scary camp

MORPHOLOGY

Comma-shaped | S-shaped | "Sea gull wing" shape

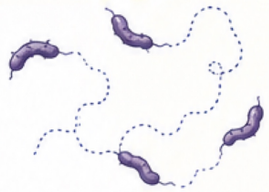
Gram-negative non-spore-forming rod

Single polar flagellum at one or both ends

SELECTIVE MEDIA EXAMPLES

Campy-Blood agar | Skirrow's medium

MOTILITY

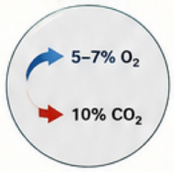


darting motility

CULTURE, BIOCHEMICAL IDENTIFICATION, AND DIAGNOSIS

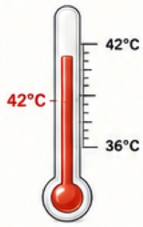
MICROAEROPHILIC ATMOSPHERE

Campylobacter species require a microaerobic atmosphere containing reduced O_2 of 5–7% and increased 10% CO_2 for optimal growth.



TEMPERATURE

Most campylobacters grow best at 42°C, although growth can be seen between 36°C and 42°C; primary plates for *C. jejuni* isolation should be incubated at 42°C.



SELECTIVE MEDIA (Skirrow's medium)

Skirrow's medium contains vancomycin, polymyxin B, and trimethoprim to inhibit growth of other bacteria.

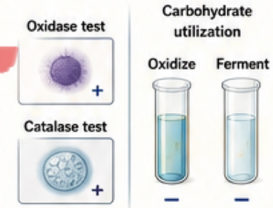
Vancomycin | Polymyxin B | Trimethoprim

COLONY MORPHOLOGY

Colonies are generally colorless or gray, may be watery and spreading or round and convex, and hemolysis on blood-containing agar media is not observed.

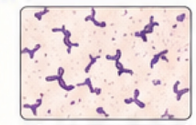
BIOCHEMICAL IDENTIFICATION

C. jejuni and *C. coli* are positives for both oxidase and catalase; campylobacters do not oxidize or ferment carbohydrates.



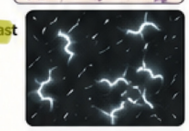
MICROSCOPY

Gram-stained smears may show typical "gull wing"–shaped rods.



Dark-field or phase-contrast microscopy may show darting motility.

حركة عشوائية



DEFINITIVE DIAGNOSIS

Culture on selective media is the definitive test to diagnose *C. jejuni* enteritis.

HIPPURATE HYDROLYSIS TEST

A positive hippurate hydrolysis test distinguishes *C. jejuni* from other Campylobacter specie.

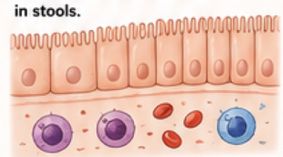
DISEASE, TREATMENT, AND EPIDEMIOLOGY

TRANSMISSION

Infection is acquired by the oral route from contaminated food, drink, poultry, or contact with infected animals.

PATHOGENESIS

Organisms multiply in the small intestine, invade the epithelium, and cause inflammation with red and white blood cells in stools.

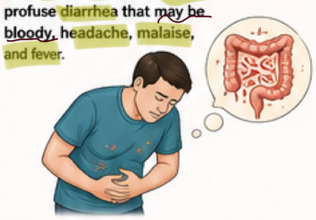


CLINICAL DISEASES

C. jejuni and *C. coli* most commonly cause gastroenteritis; *C. fetus* causes bacteremia and extraintestinal infections in pregnant women and immunocompromised patients.

GASTROENTERITIS (*C. jejuni*)

C. jejuni gastroenteritis presents with crampy abdominal pain, profuse diarrhea that may be bloody, headache, malaise, and fever.



COURSE OF ILLNESS

Illness is usually self-limited for 5–8 days; recurrence occurs in about 5–10% of patients.

TREATMENT

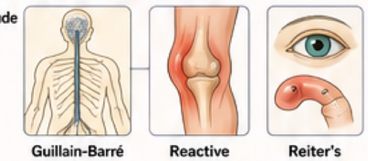
Most cases resolve without antimicrobial therapy, but macrolides, especially erythromycin, may be used and shorten fecal shedding.

Erythromycin

POSTDIARRHEAL COMPLICATIONS

Postdiarrheal complications include Guillain-Barré syndrome, reactive arthritis, and Reiter's syndrome.

can't see, pee, climb a tree



Guillain-Barré syndrome | Reactive arthritis | Reiter's syndrome

RESERVOIRS

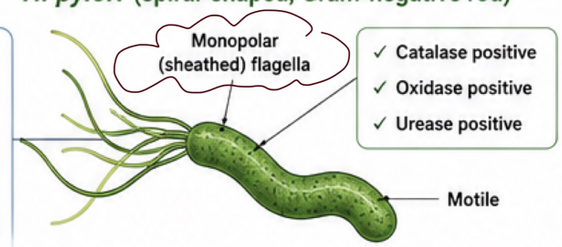
Reservoirs include poultry, cattle, sheep, pigs, birds, and pet dogs depending on the species.

Poultry | Cattle | Sheep | Pigs | Birds | Pet dogs

Helicobacter pylori

- *H. pylori* is a spiral-shaped, Gram-negative rod.
- It is catalase-positive, oxidase-positive, and urease-positive.
- It is motile and has single and/or multiple monopolar, typically sheathed flagella.
- Humans are the primary host-reservoir for *H. pylori*.
- It is associated with antral gastritis, duodenal peptic ulcer disease, gastric ulcers, gastric adenocarcinoma, and gastric MALT lymphoma.

H. pylori (spiral-shaped, Gram-negative rod)



Primary host-reservoir: Humans

IDENTIFICATION

- *H. pylori* can be isolated from gastric biopsy specimens.

- Culture requires 37°C, a microaerophilic and humid atmosphere, and usually 3–6 days of incubation.

- If culture is negative, incubation up to 14 days may be necessary.

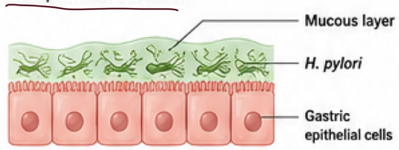
- Primary isolation uses enriched media with blood/blood products, such as chocolate agar, or antibiotic-containing media such as Skirrow's medium.

- Colonies on blood agar range from gray to translucent.

- Urease activity breaks down urea into ammonia and CO_2, helping neutralize gastric acid.

PATHOGENESIS AND VIRULENCE FACTORS

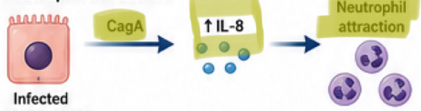
- *H. pylori* colonizes gastric-type epithelial cells and is found deep in the mucous layer near the epithelial surface.



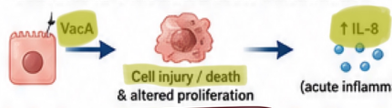
- Flagella-mediated motility allows movement through gastric mucus toward the epithelium.

- Tissue damage is related to release of mucinase, phospholipase, neutrophil-activating protein A, heat shock protein 60, cytotoxin-associated gene A protein (CagA), and vacuolating cytotoxin A (VacA).

- CagA induces IL-8 production, leading to neutrophil attraction.

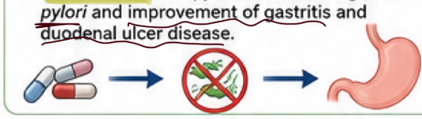


- VacA affects cell death and proliferation and activates IL-8 mediated acute inflammation.



- Histology shows acute and chronic inflammation with polymorphonuclear and mononuclear cell infiltrates.

- Antimicrobial therapy results in clearing of *H. pylori* and improvement of gastritis and duodenal ulcer disease.



CLINICAL FINDINGS

- Acute infection causes an upper gastrointesitnal illness with nausea and pain; vomiting and fever may also occur.

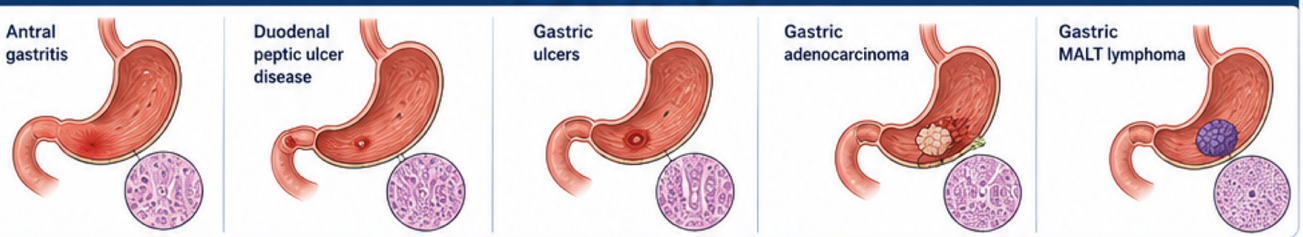
- Symptoms last less than 1 week to 2 weeks.

- After acute infection, *H. pylori* colonization may persist for years, decades, or lifelong.

- About 90% of duodenal ulcer patients and 50–80% of benign gastric ulcer patients have *H. pylori* infection.

- Long-lasting colonization is associated with chronic gastritis, intestinal metaplasia, atrophic gastritis, and gastric adenocarcinoma.

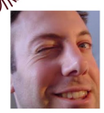
ASSOCIATED DISEASES



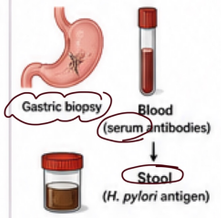
Key Point: *H. pylori* is a major cause of chronic gastritis and peptic ulcer disease and is strongly associated with gastric cancer and MALT lymphoma. Early diagnosis and appropriate antimicrobial therapy are essential.

DIAGNOSIS

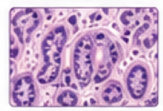
- Gastric biopsy specimens are used for histology or culture; blood is used for serum antibodies, and stool for *H. pylori* antigen detection.
- Histology is generally more sensitive than culture.
- Hematoxylin and eosin stains show acute/chronic gastritis.
- Giemsa, silver stains, or immunohistochemical stains show the curved or spiral-shaped organisms.
- Culture is used mainly when patients are not responding to treatment and antimicrobial susceptibility testing is needed.



Specimens



Histology (more sensitive than culture)



Stains for *H. pylori*

Culture (when treatment fails)

SEROLOGY AND OTHER TESTS

- IgG antibodies confirm exposure, but titers do not correlate with disease severity.
- IgM antibodies disappear rapidly and are of little diagnostic value.
- IgA and IgG persist even after eradication therefore antibody testing has limited value for active infection or cure.

Antibody Response Over Time

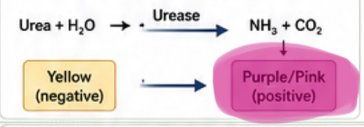
- Histology of gastric biopsy has 95–100% sensitivity and specificity.

Histology (Gold Standard)

Sensitivity	Specificity
95–100%	95–100%

- Rapid urease test detects urease activity within 1–2 hours by pH color change.

Rapid Urease Test (1–2 hours)



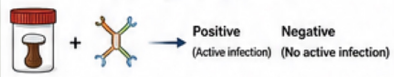
- Urea breath test detects labeled CO_2 after ingestion of ^{13}C- or ^{14}C-labeled urea; sensitivity and specificity are 94–98%.

Urea Breath Test

Sensitivity & Specificity 94–98%

- Stool antigen detection by ELISA diagnoses active infection and is useful as a test of cure.

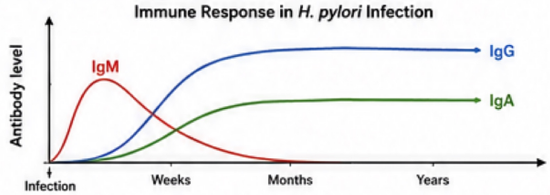
Stool Antigen Test (ELISA)



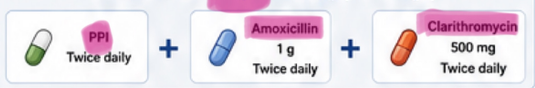
IMMUNITY AND TREATMENT

- Infection induces IgM, followed by persistent IgG and IgA in chronic infection.

Immune Response in *H. pylori* Infection



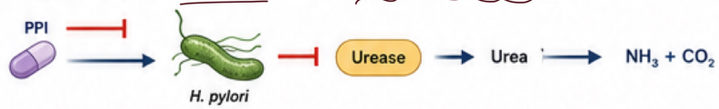
Recommended Triple Therapy (7–14 days)



Alternative Quadruple Therapy (10–14 days)

- Fourteen-day regimens eradicate *H. pylori* in 70–95% of patients.

- PPIs directly inhibit *H. pylori* and act as potent urease inhibitors.



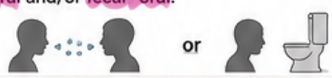
- Recurrent or persistent infection may require tailored therapy for antimicrobial-resistant strains.

Tailored Therapy Based on Susceptibility

EPIDEMIOLOGY AND CONTROL

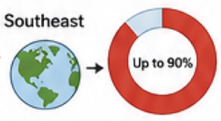
- Humans are likely the primary, if not sole reservoir for *H. pylori*.

- Transmission is mainly oral–oral and/or fecal–oral.



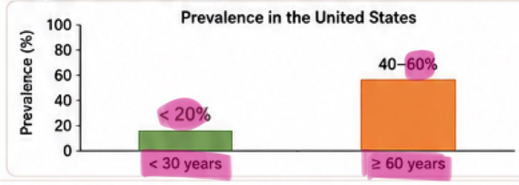
- Infection is usually acquired in early childhood and may persist lifelong without appropriate antibiotics.

- Prevalence is higher in developing countries; in Southeast Asia, adult prevalence may reach up to 90%.



- In the United States, prevalence is fewer than 20% in persons younger than 30 years, increasing to 40–60% in those aged 60 years and older.

Prevalence in the United States



- Person-to-person spread is supported by intrafamilial clustering; rare transmission occurs via improperly cleaned endoscopes.