Practical lesson 13
Microbiological diagnosis of gastrointestinal infections (genus *Escherichia, Salmonella, Shigella, Vibrio, Campylobacter, Helicobacter*)

Enterobacteriaceae

ESCHERICHIA COLI

Biology and Virulence

Gram-negative, facultative anaerobic rods

Fermenter; oxidase negative

Lipopolysaccharide consists of outer somatic O polysaccharide, core polysaccharide (common antigen), and lipid A (endotoxin)

Epidemiology

Most common aerobic gram-negative rods in the gastrointestinal tract

Most infections are endogenous (patient's microbial flora), although strains causing gastroenteritis are generally acquired exogenously

Diagnosis

- Organisms grow rapidly on most culture media
- Enteric multiplex NAATs considered gold standard diagnostic

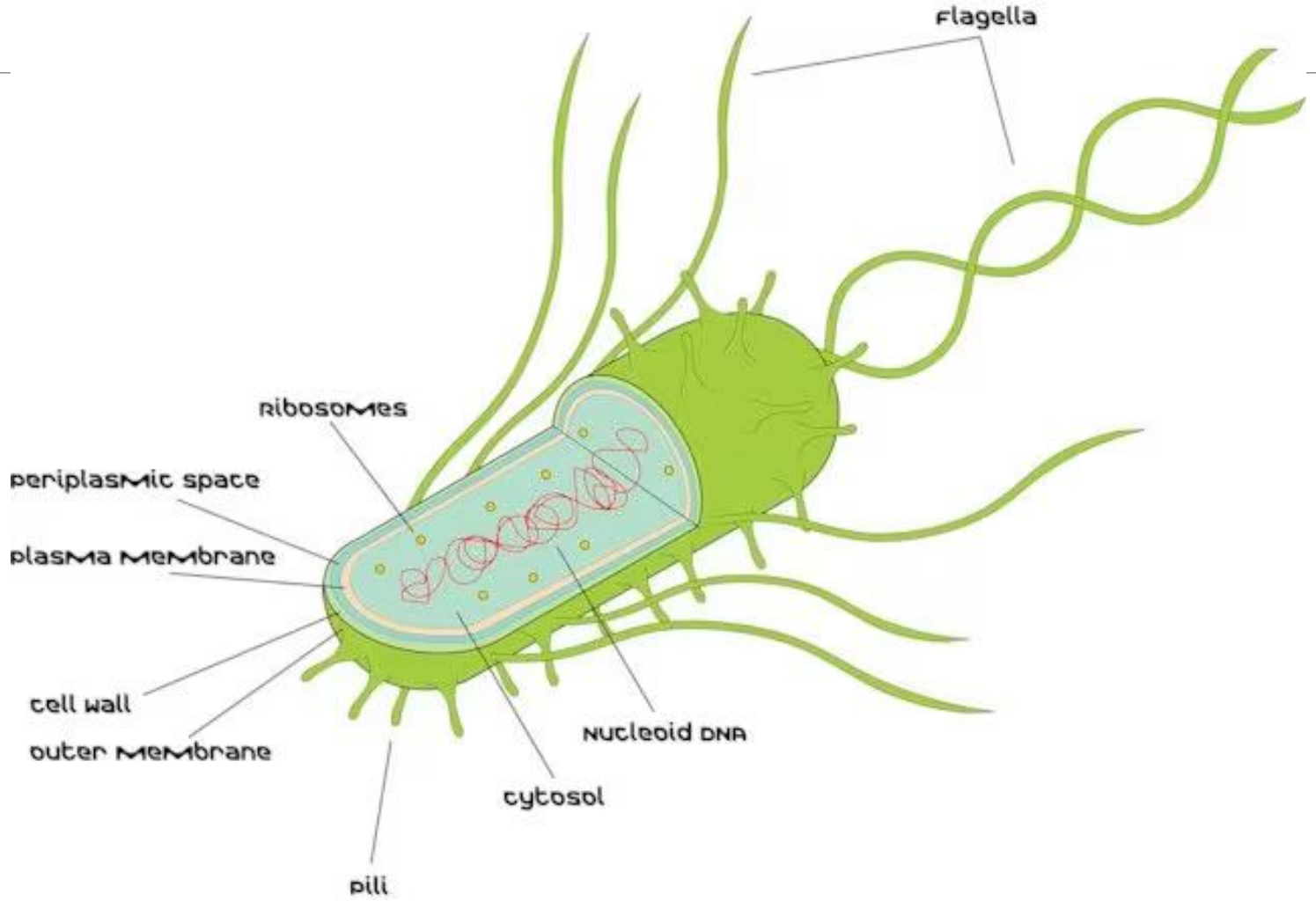
Diseases

At least five different pathogenic groups cause gastroenteritis: EAEC, EIEC, EPEC, ETEC, and STEC

Most cause diseases in developing countries, although STEC is an important cause of hemorrhagic colitis and hemolytic uremic syndrome

Extraintestinal disease includes bacteremia, neonatal meningitis, urinary tract infections, and intraabdominal infections

Escherichia coli



Treatment, Prevention, and Control

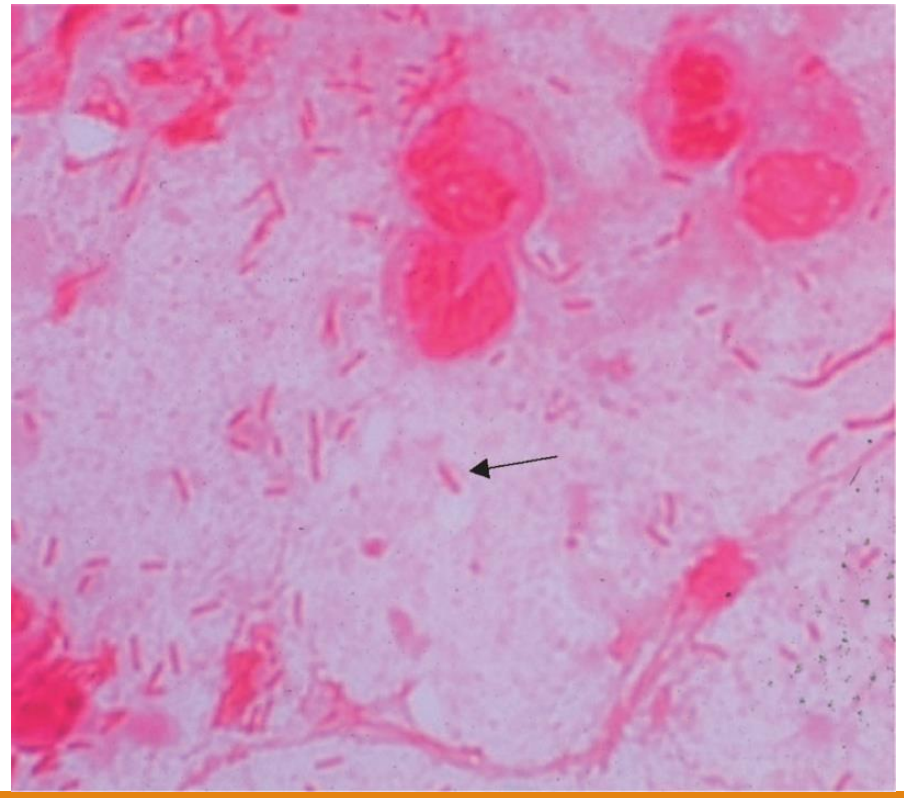
Enteric pathogens are treated symptomatically unless disseminated disease occurs

Antibiotic therapy is guided by in vitro susceptibility tests; increased resistance to penicillins and cephalosporins mediated by ESBLs

Appropriate infection-control practices are used to reduce the risk of nosocomial infections (e.g., restricting use of antibiotics, avoiding unnecessary use of urinary tract catheters)

Maintenance of high hygienic standards to reduce the risk of exposure to gastroenteritis strains

Proper cooking of beef products to reduce risk of STEC infections



SALMONELLA

Trigger Words Gastroenteritis, enteric fever, antibiotic treatment

Biology and Virulence

Gram-negative, facultative anaerobic rods

Fermenter; oxidase negative

Lipopolysaccharide consists of outer somatic O polysaccharide, core polysaccharide (common antigen), and lipid A (endotoxin)

More than 2500 O serotypes

Virulence: refer to Box 25.2; tolerant of acids in phagocytic vesicles

Can survive in macrophages and spread from the intestine to other body sites

Epidemiology

Most infections are acquired by eating contaminated food products (poultry, eggs, and dairy products are the most common sources of infection)

Direct fecal-oral spread in children

Salmonella Typhi and *Salmonella Paratyphi* are strict human pathogens (no other reservoirs); these infections are passed person to person; asymptomatic long-term colonization occurs commonly

Individuals at risk for infection include those who eat improperly cooked poultry or eggs, patients with reduced gastric acid levels, and immunocompromised patients

Infections occur worldwide, particularly in the warm months of the year

Diseases

Diseases: enteritis (fever, nausea, vomiting, bloody or nonbloody diarrhea, abdominal cramps); enteric fever (typhoid fever,

paratyphoid fever); bacteremia (most commonly seen with *Salmonella* serotype Typhi, *Salmonella* serotype Paratyphi, *Salmonella* serotype Choleraesuis); asymptomatic colonization (primarily with *Salmonella* Typhi and *Salmonella* Paratyphi)

Diagnosis

- Isolation from stool specimens requires use of selective media
- Enteric multiplex NAATs considered gold standard diagnostic

Treatment, Prevention, and Control

Antibiotic treatment not recommended for enteritis because this may prolong the duration of disease

Infections with *Salmonella* Typhi and *Salmonella* Paratyphi or disseminated infections with other organisms should be treated with an effective antibiotic (selected by in vitro susceptibility tests); fluoroquinolones (e.g., ciprofloxacin), chloramphenicol, trimethoprim sulfamethoxazole, or a broad-spectrum cephalosporin may be used

Most infections can be controlled by proper preparation of poultry and eggs (completely cooked) and avoidance of contamination of other foods with uncooked poultry products

Carriers of *Salmonella* Typhi and *Salmonella* Paratyphi should be identified and treated

Vaccination against *Salmonella* Typhi can reduce the risk of disease for travelers into endemic areas

EAEC, Enteroaggregative *E. coli*; *EIEC,* enteroinvasive *E. coli*; *EPEC,* enteropathogenic *E. coli*; ESBL, extended-spectrum _-lactamase; *ETEC,* enterotoxigenic *E. coli*; *NAAT,* nucleic acid amplification test; *STEC,* Shiga toxin–producing *E. coli*.

Important Enterobacteriaceae

Salmonella on SS Agar

Shigella on SS Agar

SHIGELLA

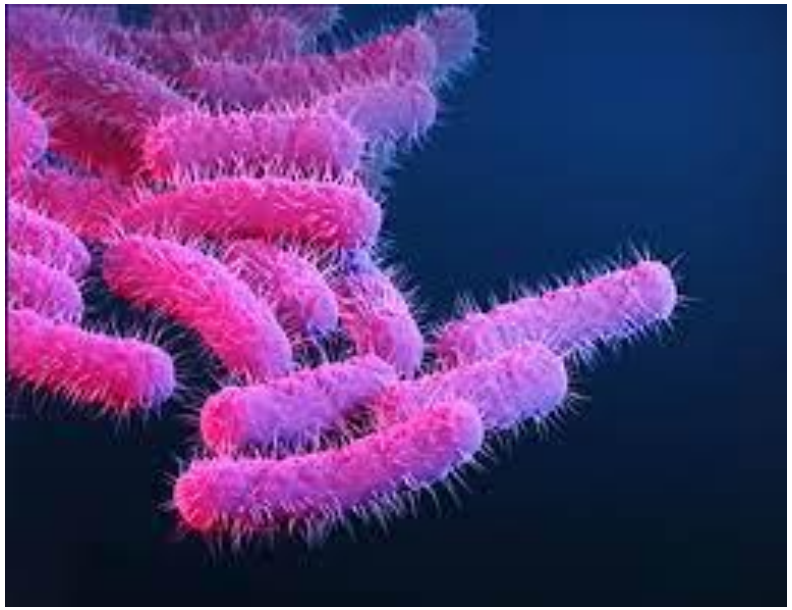
Shigella species cause enterocolitis, Enterocolitis caused by Shigella is otten called bacillary dysentery. The term dysentery reters to blood diarrhea.

Important Properties

Shigellae are non-lactose-termenting, gram-negative rods that can be distinguished from salmonellae by three crite. ria: they produce no gas from the fermentation of glucose they do not produce H.S, and they are nonmotile. All shigelhe have • antigens Dolysaccharide in their cell walls, and these antigens are used to divide the genus into four groups: A, B, C, and D.

Clinical Findings

Alter an incubation period of I to 4 days, symptoms begin with lever and abdomina cramps. tollowed by diarrhea. which may be watery at first but later contains blood and mucus. The disease varies from mild to severe depending on two major factors: the species of Shigella and the age of the patient, with young children and elderly people being the most severely affected. The diarrhea frequently resolves in 2 or 3 days; in severe cases, antibiotics can shorten the course. Serum agglutinins appear atter recovery bus are not protecave because the organism does not enter the blood.



Laboratory Diagnosis

Shigellae form non-lactose-termenting (colorless) colonies on MacConkeys or EMB agar. On TSI agar, they cause an alkaline slant and an acid butt, with no gas and no H2S Confirmation of the organism as Shigella and determina tion of its group are done by slide agglutination.

One important adjunct to laboratory diagnosis is a methylene blue stain of a fecal sample to determine whether neutrophils are present. If they are found, an invasive organism such as Sigella. Salmonella. or Campylobacter is involved rather than a toxin-producing organism such as V. cholerae, E. coli, or Clostridium perfringens.

Treatment

The main treatment for shigellosis is fluid and electrolyte replacement. In mild cases, no antibiotics are indicated. In severe cases, a fluoroquinolone (e.g., ciprofloxacin) is the drug of choice, but the incidence of plasmids conveying multiple drug resistance is high enough that antibiotic sensitivity tests must be performed. Trimethoprim-sulfamethoxazole is an alternative choice. Antiperistaltic drugs are contraindicated in shigellosis, because they prolong the fever, diarrhea, and excretion of the organism.

Prevention

Prevention of shigellosis is dependent on interruption of fecal-oral transmission by proper sewage disposal, chlorination of water, and personal hygiene (handwashing by food handlers). There is no vaccine, and prophylactic antibiotics are not recommended.

VIBRIO

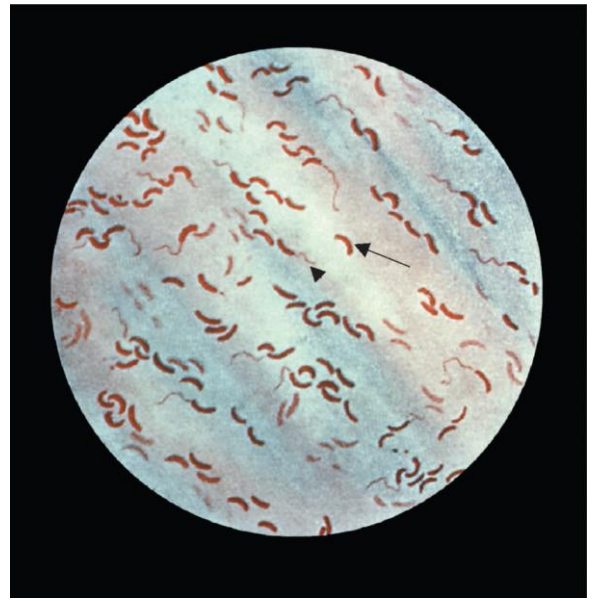
Diseases

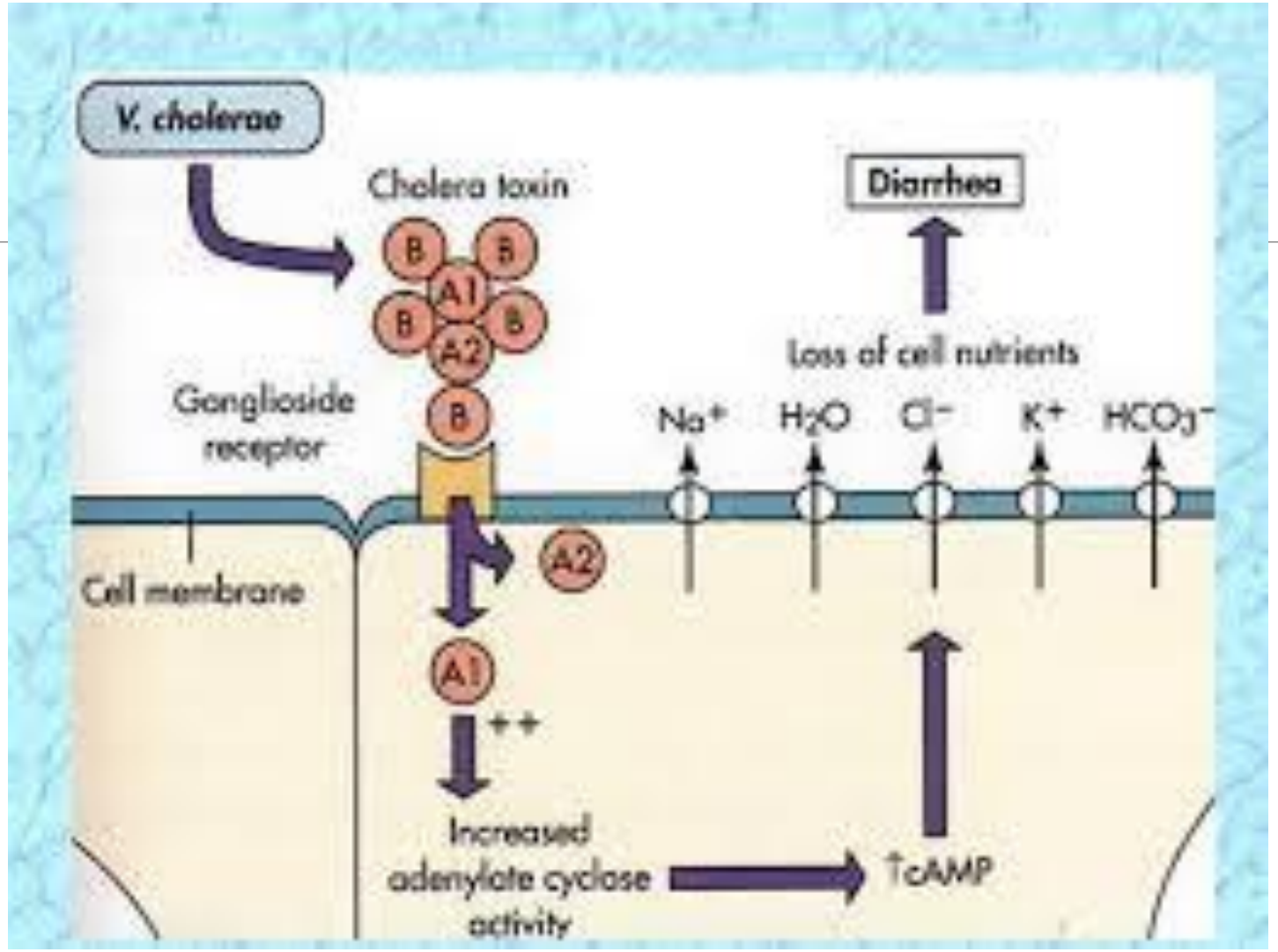
Vibrio cholera, the major pathogen in this genus, is the cause of cholera. Vibrio parahaemolyticus causes diarrhea associated with eating raw or improperly cooked seafood.

Vibrio vulnificus causes cellulitis and sepsis.

Important Properties

Vibrios are curved, comma-shaped, gram-negative rods . V. cholerae is divided into two groups according to the nature of its O cell wall antigen. Members of the 01 group cause epidemic disease, whereas non-01 organisms either cause sporadic disease or are nonpatho-gens.




V. cholerae
Cholera toxin
B
B
A1
B
B
A2
B
Ganglioside receptor
Cell membrane
A2
A1
++
Increased adenylate cyclase activity
↑cAMP
Diarrhea
Loss of cell nutrients
Na+
H2O
Cl−
K+
HCO3−

CAMPYLOBACTER

Diseases

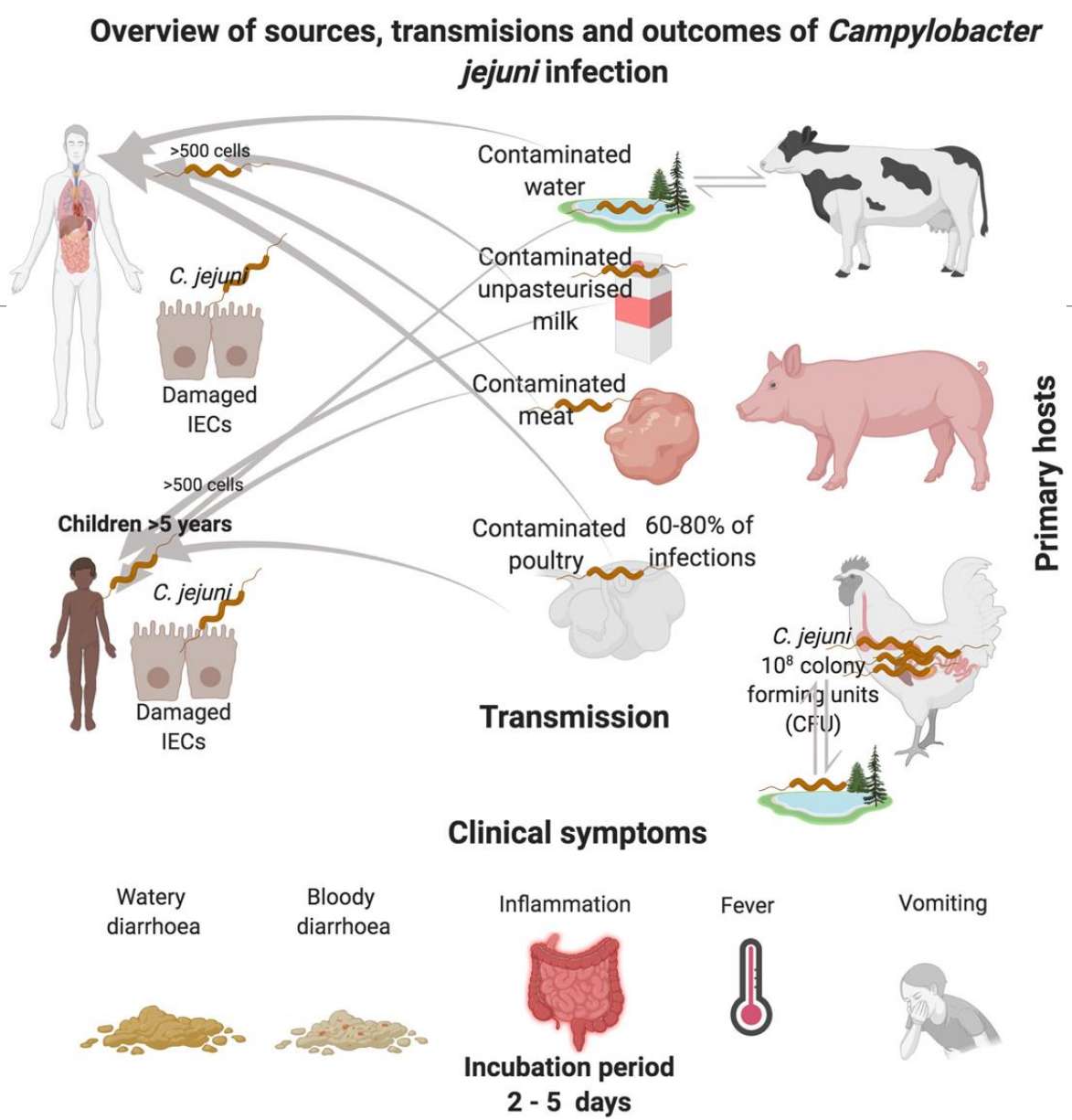
Campylobacter jejuni is a frequent cause of enterocolitis, especially in children. C. jejuni infection is a common antecedent to Guillain-Barré syndrome. Other Campylobacter species are rare causes of systemic infection, particularly bacteremia.

Important Properties

Campylobacters are curved, gram-negative rods that appear either comma- or S-shaped. They are microaerophilic, growing best in 5% oxygen rather than in the 20% present in the atmosphere. C. jejuni grows well at 42°C, whereas Campylobacter intestinalis4 does not—an observation that is useful in microbiologic diagnosis.

Pathogenesis & Epidemiology

Domestic animals such as cattle, chickens, and dogs serve as a source of the organisms for humans. Transmission is usually fecal–oral. Food and water contaminated with animal feces are the major sources of human infection. Foods, such as poultry, meat, and unpasteurized milk, are commonly involved. Puppies with diarrhea are a common source for children. Human-to-human transmission occurs but is less frequent than animal-to-human transmission. Campylobacter jejuni is a major cause of diarrhea in the United States; it was recovered in 4.6% of patients with diarrhea, compared with 2.3% and 1% for Salmonella and Shigella, respectively. Campylobacter jejuni is the leading cause of diarrhea associated with consumption of unpasteurized milk.


Overview of sources, transmisions and outcomes of Campylobacter jejuni infection
>500 cells
C. jejuni
Damaged IECs
>500 cells
Children >5 years
C. jejuni
Damaged IECs
Contaminated water
Contaminated unpasteurised milk
Contaminated meat
Contaminated poultry
60-80% of infections
Transmission
C. jejuni
10⁸ colony forming units (CFU)
Primary hosts
Clinical symptoms
Watery diarrhoea
Bloody diarrhoea
Inflammation
Fever
Vomiting
Incubation period
2 - 5 days

HELİCOBACTER

Diseases

Helicobacter pylori causes gastritis and peptic ulcers. Infection with H. pylori is a risk factor for gastric carcinoma and is linked to mucosal-associated lymphoid tissue (MALT) lymphomas.

Important Properties

Helicobacters are curved gram-negative rods similar in appearance to campylobacters, but because they differ sufficiently in certain biochemical and flagellar characteristics, they are classified as a separate genus. In particular, helicobacters are strongly urease-positive, whereas campylobacters are urease-negative.

Pathogenesis & Epidemiology

Helicobacter pylori attaches to the mucus-secreting cells of the gastric mucosa. The production of large amounts of ammonia from urea by the organism's urease, coupled with an inflammatory response, leads to damage to the mucosa. Loss of the protective mucus coating predisposes to gastritis and peptic ulcer . The ammonia also neutralizes stomach acid, allowing the organism to survive. Epidemiologically, most patients with these diseases show H. pylori in biopsy specimens of the gastric epithelium. The natural habitat of H. pylori is the human stomach, and it is probably acquired by ingestion. However, it has not been isolated from stool, food, water, or animals. Personto-person transmission probably occurs because there is clustering of infection within families. The rate of infection with H. pylori in developing countries is very high—a finding that is in accord with the high rate of gastric carcinoma in those countries. MALT lymphomas are B-cell tumors located typically in the stomach, but they occur elsewhere in the gastrointestinal tract as well. Helicobacter pylori is often found in the MALT lesion, and the chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually a B-cell lymphoma. Antibiotic treatment directed against the organism often causes the tumor to regress.

Clinical Findings

Gastritis and peptic ulcer are characterized by recurrent pain in the upper abdomen, frequently accompanied by bleeding into the gastrointestinal tract. No bacteremia or disseminated disease occurs.

Laboratory Diagnosis

The organism can be seen on Gram-stained smears of biopsy specimens of the gastric mucosa. It can be cultured on the same media as campylobacters. In contrast to C. jejuni, H. pylori is urease-positive. Urease production is the basis for a noninvasive diagnostic test called the "urea breath" test. In this test, radiolabeled urea is ingested. If the organism is present, urease will cleave the ingested urea, radiolabeled CO_2 is evolved, and the radioactivity is detected in the breath. A test for Helicobacter antigen in the stool can be used for diagnosis and for confirmation that treatment has eliminated the organism. The presence of IgG antibodies in the patient's serum can also be used as evidence of infection.