- **Discuss the disease produced by Vibrio cholerae with emphasis on its prevention and diagnosis**

- **Describe characteristics and importance of microaerophilic members of group Campylobacter and Helicobacter.**
-

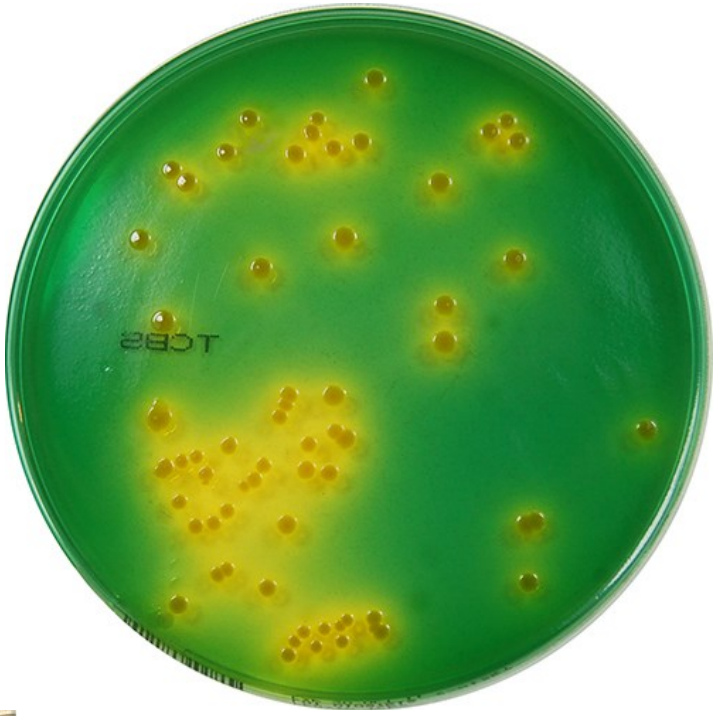
- Belong to the Family – Vibrionaceae
- Gram-negative curved or comma shaped bacilli
- Highly motile by a single polar flagellum
- Non spore formers
- Non capsulated
- Facultative anaerobes
- Possess both H & O antigens

•

Identification

- Vibrios are highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum

- Grow on variety of simple media including:
  - MacConkey's agar
  - TCBS (Thiosulfate Citrate Bile salts Sucrose) agar



- *V. cholerae* grow without salt
  - Most other vibrios are halophilic-salt loving

On MacConkey agar *V. cholerae* produces small lactose fermenting colonies.

- *V. cholerae*
- *V. parahaemolyticus*
- *V. vulnificus*

- All *Vibrio* spp. can survive and replicate in contaminated waters with increased salinity and at temperatures of 10-30^{o}C

- Pathogenic *Vibrio* spp. appear to form symbiotic (?) associations with chitinous shellfish which serve as an important and only recently recognized reservoir

- Asymptomatically infected humans also serve as an important reservoir in regions where cholera is endemic

Introduction

- Causes cholera
- Classified as category B bioterrorism agent- water supply threat (CDC)
- Transmitted by ingestion of contaminated water (sewage contaminated water is the primary source)
- Poor sanitation practices are the source of outbreaks

Epidemiology

- Responsible for seven global pandemics over the past two centuries
- Common in India, Sub-Saharan Africa, Southern Asia
- Very rare in industrialized countries

Strains Causing Epidemics

- 2 main serogroups carry set of virulence genes necessary for pathogenesis
- O1
  - Classical: 1 case per 30-100 infections
  - El Tor: 1 case per 2-4 infections
- O139
  - Contained in India, Bangladesh

Transmission

- Contaminated food or water
  - Inadequate sewage treatment
  - Lack of water treatment
  - Improperly cooked shellfish
  - Wash fruits/vegetables with untreated water

- Transmission by casual contact unlikely

Pathogenesis: Overview

- To establish disease, V. cholerae must be ingested in contaminated food or water and survive passage through the gastric barrier of the stomach
- On reaching the lumen of the small intestine, penetrates the mucous layer and establish contact with the epithelial cell layer
- High Infectious dose is required to elicit an infection

Pathogenesis

- Virulence factors
  - Bacteria adheres to the epithelia cells of gastric intestinal mucosa via pilli
  - survive the acidic conditions of the human stomach
  - Production of proteases (mucinase) which dissolves protective glycoprotein coating cells
  - High motility
  - Ability to produce an enterotoxin-cholera toxin (CT)

How Does Cholera Toxin Work?

- Inactivates GTPase function of G-protein coupled receptors in intestinal cells
- G proteins stuck in “On” position
- 100 fold increase in cAMP
- Activation of ion channels
- Ions flow out and water follows

- Incubation period: 2-3 days
- High infectious dose: >10^8 CFU
  - 10^3 -10^5 CFU with achlorhydria or hypochlorhydria (lack of or reduced stomach acid)
- Abrupt onset of vomiting and life-threatening watery diarrhea (15-20 liters/day)
- As more fluid is lost, feces-streaked stool changes to rice-water stools:
  - Colorless
  - Odorless
  - No protein
  - Speckled with mucus

People Most at Risk

- People with low gastric acid levels
  - Children: 10x more susceptible than adults
  - Elderly
- Blood types
  - O>> B > A > AB (O more susceptible—allows bacteria to adhere to gut lining???)

Diagnosis: Visible Symptoms

- Decreased skin turgor
- Sunken eyes, cheeks
- Almost no urine production
- Dry mucous membranes
- Watery diarrhea consists of:
  - fluid *without* RBC, proteins
  - electrolytes
  - enormous numbers of vibrio cholera (10^7 vibrios/mL)

Outline of laboratory isolation and identification

- **Specimen; ??????**
- **Cary-Blair transport medium**
- **Microscopy; Wet preparation -darting motility**

Enrichment medium - Alkaline peptone water for 4-6hrs

- **SubCulture on TCBS (thiosulphate citrate bile salt sucrose) in air, at 37°C, for 18-24hrs**

  Colonial morphology; yellow colonies
- **Gram stain of colony; ????**
- **Biochemical test; Oxidase positive**

Other tests

- **Toxin assays**
- **Dipstick**
- **PCR**

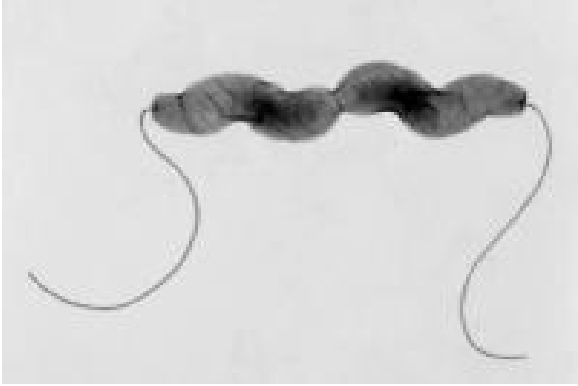
Treatment

Even before identifying cause of disease, rehydration therapy must begin Immediately because death can occur within hours

- Oral rehydration
- Intravenous rehydration
- Antimicrobial therapy

Campylobacter

Campylobacter

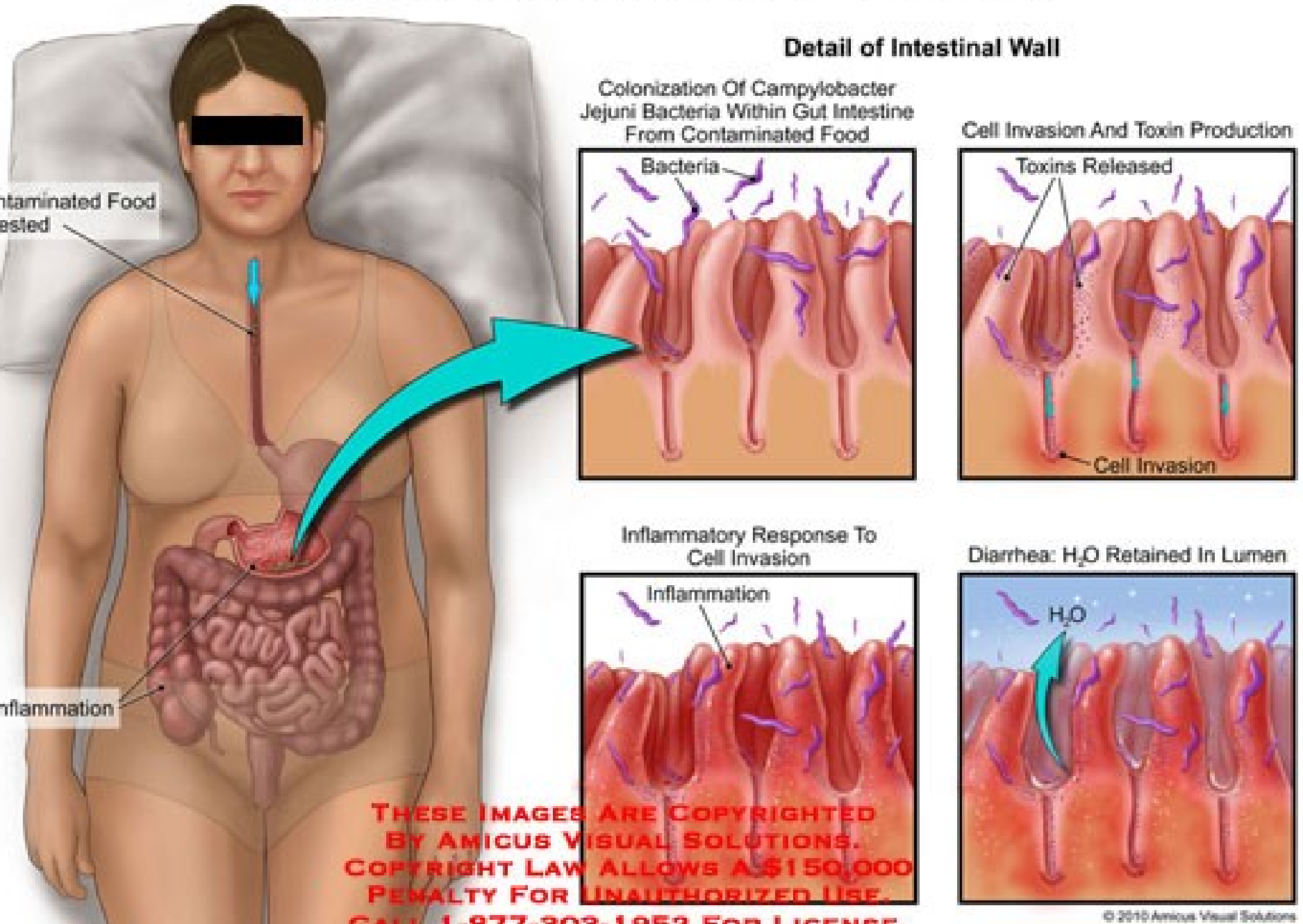


- Gram negative curved rods
  - Often attached in pairs giving a “seagull” appearance
- Motile by polar flagella; darting motility in corkscrew fashion
  - Facilitate penetration and colonization of mucosal environment
- Species; ***Campylobacter jejuni*** – causes 80-90% of all illnesses

Source

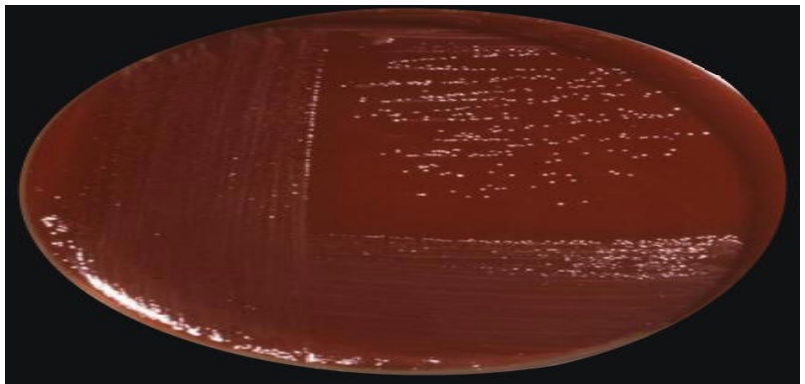
- ***C. jejuni***
  - commonly associated with poultry,
  - naturally colonises the digestive tract of many bird species
  - also common in cattle
- **Transmission**
  - Raw or undercooked food products
  - Through direct contact with infected animals

Campylobacter Jejuni Food Poisoning



Laboratory diagnosis

- Specimen- stool, blood, etc
- Wet preparation of stool: dark-field microscopy observe darting motility
- Culture on **Skirrow's medium**
  - Incubate in microaerophilic conditions at 42°C, for up to 48 hours
  - Gram stain – Gram negative comma- or S-shaped rods
  - Oxidase positive



Treatment

- Rehydration - usually self-limiting
- Severe dysentry – erythromycin, ciprofloxacin
- Antimotility agents not recommended

Helicobacter

Dr Barry Marshal was convinced that H. pylori bacteria caused stomach ulcers, but no one believed him. Since it was illegal to test his theory on humans, he drank the bacteria himself, developed ulcers within days, treated them with antibiotics and went on to win a Nobel prize.

Weird World

Helicobacter

- Colonizes stomach ≈ 50% of world population
- *H. pylori* is contagious, although the exact route of transmission is not known.
- Main risk factor for;
  - Gastric & duodenal ulceration
  - Stomach cancer
  - Gastric MALT (mucosa-associated lymphoid tissue) lymphoma

Characteristics

- Gram negative curved rod
- Microaerophilic: requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere
- Motile
- Produces oxidase, mucinase and urease enzymes

Virulence Factors

- **Corkscrew motility** enables penetration into mucosal layer
- **Adhesins:** which help it adhere to epithelial cells
- **Mucinase:** Degrades gastric mucus; Localized tissue damage
- **Urease** converts urea into ammonia
  - Neutralize the local acid environment
  - Localized tissue damage
- Vacuolating cytotoxin (VacA) – apoptosis in eukaryotic cells generating large cytoplasmic vacuoles – epithelial cell damage

Pathogenesis

Organism attached to the mucus – secreting cells of gastric mucosa

⇩

Production of large amount of ammonia from urea by bacterial urease

⇩

Ammonia neutralizes HCl in stomach

⇩

Organism survival

⇩

Plus inflammatory response

⇩

Damage to the mucus

⇩

Gastritis + peptic ulcers

Laboratory diagnosis

Non-invasive

- Blood antibody test- IgG detection
- Stool antigen test- detection of specific antigens in the stools
- carbon urea breath test

Invasive - most reliable

- Gastric biopsy
  - Urease positive
  - Culture on Helicobacter Agar- takes 3-5days
    - –Oxidase, urease positive

Treatment of H. pylori infection

First line treatment	
Standard triple therapy	"legacy triple therapy"
Sequential therapy	"five plus five day therapy"
Concomitant therapy	"non-bismuth quadruple therapy"
Sequential-concomitant therapy	"hybrid therapy"
Bismuth quadruple therapy	underutilized in practice
Second line treatment (one treatment failure)	
Levofloxacin triple therapy	
Third line treatment (at least 2 treatment failures)	
Culture-guided therapy	Recommended
High-dose dual PPI therapy	Empirical
Furazolidone quadruple therapy	Empirical
Rifabutin-based triple therapy	Empirical – Last resort

Malfertheiner P et al. Gut 2012 ; 61: 646 – 664.

CONCLUSIONS