I. **Vibrio cholera**

A. Structure

1. Morphology/Staining
   a. Curved, gram-negative rod found in salt water

2. Structural components
   a. Single polar flagellum – motility
   b. Tcp pili – Toxin Coregulated Pili – N-methyl phenylalanine type, like gonococcus and other organisms
   c. Lipopolysaccharide outer membrane with O antigen

3. Exotoxin – RESPONSIBLE FOR THE DISEASE CHOLERA
   a. AB-type ribosylating toxin
   b. B (5 subunits) binds to GM1 ganglioside, a sialic acid-containing oligosaccharide localized in lipid rafts on cell surface (see pathogenesis)
   c. A is endocytosed and travels to ER

B. Growth characteristics

1. Grows in alkaline conditions, pH 8.0-8.5
   a. Inhibits other gram negative bacteria

2. Facultative anaerobe

3. **Oxidase positive**

4. Separated into serogroups and biotypes
   a. Serogrouping by O-antigen on outer membrane polysaccharide
      i. More than 200 serogroups (O1-O200) of which only O1 and O139 can cause clinical cholera
   b. Biotypes by reactions in laboratory
      i. Important biotypes are **classical** and **El tor**

Until 1993, epidemics of cholera were caused by serogroup O1. Two important biotypes, **classical** and **El-tor** were responsible for most pandemics. **El-tor** appeared in 1905 and remains the current pandemic strain. In 1991 **El-tor** surfaced in South and Central America. **El-tor** strains have greater ratio of subclinical to clinical infections and survive longer in water than classical strains. In 1993, an large outbreak of fulminant cholera caused by a new serogroup O139 erupted in India and Bangladesh and SE Asia. Incidence of O193 decreasing

C. Genetics – Genes for virulence factors including the TCP pilus and cholera toxin are organized within a pathogenicity island.
1. Triggered by environmental factors (pH, temperature, osmolality), transcription of the genes is closely regulated.
2. Virulence requires this gene followed by infection with bacteriophage CTXΦ

D. Epidemiology
1. Excreted in stool→water→person by drinking, bathing or in food
2. Short lived state of hyperinfectivity – fresh stool person to person
3. Survives only a few days in environment
4. Current major cholera activity
   a. Endemic in Sub-Saharan Africa and South Asia
   b. Epidemic
      i. Haiti - ongoing since 2010, 10,000 deaths and 1 million cases
      ii. Yemen – following civil war, estimated 1 million cases by end of 2017

E. Pathogenesis of Cholera
1. Inoculum - >10^8 required to overcome gastric acid
   a. Person to person transmission uncommon
2. Motility, chemotaxis, mucinase→access the mucosa
3. Adherence – pili and other adhesins (cellular receptor unknown)
4. Cholera toxin
   a. B subunit binds to GM_1 ganglioside and is internalized
   b. A1 catalyzes ADP-ribosylation of regulatory G protein
   c. Gs regulates activation of adenylate cyclase system
      i. Normally reversible
      ii. ADP ribosylation results in irreversible activation of adenylate cyclase and continuous production of cAMP
      iii. cAMP activates protein kinase A (PKA)
      iv. PKA phosphorylates the chloride channel, CFTR
         a) Secretion of chloride and water at crypt
         b) Inhibition of uptake of Na and water at the villous tip

F. Immunity to cholera
1. Gastric acid – decreased acid associated with increased susceptibility, i.e., lower inoculum required to produce disease
2. Immunity derived principally from mucosal IgA
   a. Against lipopolysaccharide (O antigen)
   b. Against cholera toxin (less important)
3. Natural immunity provides long term protection for either O1 or O139 but not both.

G. Clinical Manifestations
1. Incubation 1-3 days
2. Abrupt onset profuse watery diarrhea and vomiting
3. No fever
4. Clear, “rice-water” stool
5. Profound dehydration hypotension acidosis death

H. Laboratory Diagnosis
1. Gram stain early – curve gram negative rods
2. Culture
   a. Grows on blood agar and all enteric media
   b. Selective media – Thiosulfate-citrate-bile salts-sucrose agar (TCBS)
   c. PCR including multiplex PCR test in use at LUMC

I. Treatment
1. Rehydration with fluid and electrolytes
   a. IV physiologic amounts of sodium and chloride plus excess bicarbonate and potassium
   b. Oral – electrolyte solutions containing glucose permit electrolyte absorption (cholera toxin does not inhibit a glucose-linked sodium absorption mechanism)
2. Antibiotics reduce toxin production and decrease transmission
   a. Azithromycin, doxycycline

J. Prevention
1. Sanitation critical (epidemics occur when catastrophe disrupts sanitation)
2. Vaccines
   a. Single dose oral live attenuated vaccine – CVC 103-HgR (Vaxchora) licensed by FDA, used in epidemics and recommended for travelers to cholera affected areas
II. CAMPYLOBACTER

A. Introduction

Campylobacter species are gram-negative rods with morphology similar to vibrios. *Campylobacter jejuni* and to a lesser extent *Campylobacter coli* are important causes of acute gastroenteritis. A newly described species, *Campylobacter upsaliensis* accounts for about 10% of campylobacter-caused gastroenteritis. *Campylobacter fetus* is a rare cause of septicemia and localized infection.

<table>
<thead>
<tr>
<th>Campylobacter Species Associated with Human Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>Campylobacter coli</em></td>
</tr>
<tr>
<td><em>Campylobacter fetus</em></td>
</tr>
<tr>
<td><em>Campylobacter upsaliensis</em></td>
</tr>
</tbody>
</table>
1. Morphology/staining
   a. Small, thin, motile gram-negative rods
   b. Pairs, seagull appearance

2. Growth characteristics
   a. Microaerophilic – reduced (5-7% O₂), increased (5-10% CO₂)
   b. Oxidase positive
   c. Biochemically inactive
   d. Grows better at 42°C than at 37°C.

C. Epidemiology
   1. Responsible for 4-30% infectious diarrhea
   2. Reservoir – GI and GU tracts of animals: sheep, cattle, chickens, pigs, birds, etc.
      a. Raw or poorly cooked chicken responsible for 50-70% of sporadic infections (found in 74% of supermarket chicken samples from study in Minnesota)
      b. Unpasteurized milk
      c. Waterborne – contaminated well or surface
   3. Person to person is rare
   4. C. upsaliensis – contact with domestic dogs

D. Pathogenesis
   1. Inoculum at least 10⁴, lower if gastric acid absent or neutralized
   2. Adhesins, toxins, toxic enzymes have been detected but role in clinical disease is not known
   3. histologic damage to mucosa of jejunum, ileum and colon
      a. Edema, hemorrhage, crypt abscesses with neutrophils, mononuclear cells and eosinophils in lamina propria
   4. Association with Giullain-Barre’ Syndrome
      a. Autoimmune inflammatory neuropathy
      b. Antigenic cross reaction between Campylobacter surface lipooligosaccharides (LOS) and peripheral nerve gangliosides
         i. Triggers demyelination and axonal degeneration
      c. Estimated to occur in 0.25-0.65/1,000 Campylobacter infections; but Campylobacter precedes at least 20-50% of GBS
   5. Associated with Reactive Arthritis

E. Clinical Manifestations
   1. Incubation 2-5 days
   2. Severe abdominal pain followed by diarrhea
3. Fever common
4. Diarrhea often with blood
5. Self-limited usually about 7 days

F. Laboratory diagnosis
   1. Culture – selective media with decreased O2 and increased CO2 at 42°C
   2. PCR is replacing culture

G. Treatment
   1. Antibiotics may shorten course but not recommended for uncomplicated infection in non-immunocompromised

H. Prevention – improved food preparation has decreased incidence
   *Clean cutting boards and wash hands*

III. Helicobacter

Helicobacter species are spiral-shaped, gram negative rods that were found in the stomachs of patients with gastritis in 1983. Though now universally acknowledged to be important causes of diseases of the stomach and small bowel, the original reports were met with skepticism throughout the medical community (*who would have thought that peptic ulcer was an infectious disease!*). The discoverers, Drs. Barry J. Marshall and J. Robin Warren were awarded the Nobel Prize in Medicine in 2005.

After ingestion, *H. pylori* colonizes, persists in and causes inflammation in the gastric mucosa. The infection is linked to gastritis, gastric and duodenal ulcer, gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) B-cell lymphoma.

A. Structure
   1. Morphology/staining
      a. Curved (sometimes spiral) gram negative rods.
      b. Highly motile, corkscrew motility.
   2. Growth characteristics
      a. Microaerophilic – like Campylobacter
      b. Oxidase positive
      c. UREASE POSITIVE
      d. Non-oxidative, non-fermentative

B. Epidemiology
   1. Acquired early in life in developing countries
a. 70%-90% of population seropositive

2. In US less than 40%

3. Humans primary reservoir – likely fecal-oral transmission

C. Pathogenesis – Enters gastric mucus, penetrates mucus, attaches to mucosa, invades epithelium, evades the immune response and PERSISTS

1. Genetic variation
   a. 32 or more outer membrane proteins include adhesins
   b. Can evade immune response by altering genetic structure by several mechanisms

2. Urease – formation of ammonia and CO2
   a. "mini alkaline environment"

3. Motility – swims through the mucus

4. Adherence by multiple surface adhesion proteins

5. Tissue damage and inflammation
   a. Vacuolating cytotoxin. VacA – penetrates epithelium and produces vacuoles
   b. Cag (Cytotoxin-associated gene) pathogenicity island – encodes 30+genes including
      i. Type IV secretion system which injects CagA and Cag E proteins into epithelial cell
      ii. CagA and CagE proteins have multiple functions
         a) Induce pro-inflammatory cytokines, especially IL-8. These promote inflammation and attract neutrophils which release proteases and reactive oxygen molecules

D. Clinical Manifestations

1. Gastritis – sense of fullness, nausea, vomiting and epigastric pain

2. Gastric and duodenal ulcer – pain and bleeding

3. Gastric carcinoma – Population studies demonstrate increased incidence ranging from 2 to 6-fold

4. Gastric mucosa-associated B-cell lymphoma – pain or discomfort, loss of appetite, weight loss and bleeding
   a. Therapy directed at H. pylori→regression of most early lesions

5. Associated with Immune Thrombocytopenia (ITP)

E. Diagnosis

1. Noninvasive
   a. Serology – cannot distinguish active from past infection
b. Urea breath test – measures ammonia after ingestion of labeled urea

c. **Stool antigen test** – monoclonal antibodies

d. PCR expensive and not widely used

2. Invasive requires endoscopy and biopsy

a. Urease test – can measure directly on biopsy tissue

b. Histology – stain organisms (Warthin-Starry silver most sensitive)

c. Culture – Difficult and cumbersome – required for antibiotic sensitivity testing

3. Treatment – Multidrug therapy required

a. Combination of proton pump inhibitor (e.g., omeprazole) and two antibiotics (amoxicillin, clarithromycin, metronidazole, tetracycline, fluoroquinolone) for 10-14 days.

b. Sequential regimens now preferred

c. Bismuth salts used in some regimens