Infectious Diarrhea

Vibrio, Campylobacter, Helicobacter

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Epidemiology Diarrhea

Morbidity and Mortality

- Population Group
  - Mortality
    - World Children*
    - USA Children
    - USA Total
  - Morbidity
    - Tropics Children
    - USA Total
    - USA Children
    - USA Day-care

- Estimated Incidence
  - Mortality
    - World Children*: 4.6-6.0 million
    - USA Children: 500
    - USA Total: 10,000
  - Morbidity
    - Tropics Children: 15-19/child-year
    - USA Total: 1.5-1.9/person-year
    - USA Children: 2-3.2/child-year
    - USA Day-care: 5/child-year

*Asia, Africa, Latin America

Epidemiology Diarrhea

Morbidity and Mortality

- 1.7 Billion Cases per year
- Second leading cause of death in children under 5
- 760,000 deaths annually
- Source – contaminated food and water
Diarrhea: Microbial Factors

- Attachment
  - Surface fimbriae (Tcp in *V. cholerae*, CFA I-IV in *E. coli*)
  - Effacing adherence
- Invasion
- Toxin production
  - Enterotoxins
  - Cytotoxins
- Invasiveness
  - *Shigella* and *E. coli*
  - *Salmonella* and *Yersinia*

Pathogenesis – Noninflammatory

No fecal WBC's, small intestine, watery diarrhea

- Toxigenic bacteria, e.g. enterotoxigenic *E. coli*, *V. cholerae*
  - Colonize small intestine (adhesin)
  - Produce toxin affecting chloride secretion and sodium absorption
- Viruses cause death of enterocytes
- Protozoa in small bowel
- Bacteria – preformed toxin in food

Diarrhea – Inflammatory

Fecal WBC’s, large intestine, low volume of stool with mucus, blood and pus

- Invade and damage intestinal mucosa causing inflammation and ulcer or…
- May produce cytotoxin
- Examples
  - Bacteria – *Shigella*, *Campylobacter*, *E. coli*
  - Protozoa – *Entamoeba histolytica*
  - Toxin – *Clostridium difficile*, enterohemorrhagic *E. coli*
Morphology and Staining of Vibrio cholerae

- Curved Gram-negative rod
- Found in salt water

Structural Composition of Vibrio cholerae

- Single polar flagellum → motility
- Tcp pili – Toxin coregulated pili
  - N-methylphenylalanine type, like gonococcus
- Lipopolysaccharide with O antigen
- Exotoxin – potent enterotoxin
  - AB type ADP-ribosylating toxin
    - B binds to GM-1 ganglioside – sialic acid containing
      linked to a ceramide lipid
    - A (A1 and A2) – A1 released from A2 and enters cell by
      translocation

Cultural Characteristics of Vibrio cholerae

- Grows in alkaline conditions, pH 8.0-9.5
  - Inhibits other gram-negative bacteria
- Facultative anaerobe
- Oxidase positive
- Classification
  - Serogrouped into >200 serogroups by O- antigen
    of LPS
    - Examples: O-1, non O-1, O-139
  - Only O-1 and O-139 cause the disease cholera
  - Biotypes by biochemical reactions
    - O-1 Classical
    - O-1 El tor
Regulation of Transcription of Virulence Factors

- Regulon control of ctx AB and as many as 20 other genes
  - Pilin biosynthesis
  - Siderophore
- Triggered by environmental factors
  - Temperature
  - pH
  - Osmolality
- Virulence requires the regulon followed by infection of bacteriophage CTXΦ which encodes for toxin genes ctxA and ctxB

Cholera Epidemiology

- Excreted in stool __water__ other person
  - Short lived state of hyperinfectivity – fresh stool person to person
- Originates in India, SE Asia → shipping lanes, trade routes, pilgrims
- Survives only few days in water
- El Tor – 1905 to present
  - Longer survival in water
  - Subclinical infections more frequent
  - Responsible for South and Central American cholera in 1990’s, Haiti in 2010
- 1993 – Serogroup O139 appeared in India

Cholera – Endemic and Epidemic Disease

- Endemic in Sub-Saharan Africa and South Asia – over 1,000,000 cases per year
- Epidemics
  - Haiti
    - Introduced by UN Peacekeepers in 2010
    - 10,000 deaths and 1 million cases
  - Yemen
    - Followed bloody civil war and ravaged infrastructure
    - Estimated to be 1,000,000 cases by end of 2017

True burden unknown but estimated several million cases per year in Africa and Asia
WHO estimates 1.4-4.3 million cases and 28,000-42,000 deaths per year
Pathogenesis of V. cholerae

- Inoculum – $10^8$ or more required
- Motility
- Chemotaxis
- Mucinase
- Adherence
- Exotoxin

Adherence of V. cholerae

- Tcp pili – Toxin coregulated pili
  - N-methylphenylalanine type
- Complex regulation – 15 genes
  - Structural
  - Regulation of expression
  - Pilus assembly and secretion
- Mucosal cell receptor not identified
- Other adhesions

Pathogenesis of Vibrio cholerae

Clemens et al. Nature Reviews Gastroenterology & Hepatology 2011; 8: 701
Electrolyte Composition of Stools in Adult Patients with Cholera

<table>
<thead>
<tr>
<th>Ion</th>
<th>mEq/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>126 ± 9</td>
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<tr>
<td>Potassium</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>47 ± 10</td>
</tr>
<tr>
<td>Chloride</td>
<td>90 ± 9</td>
</tr>
</tbody>
</table>

Mean ± S.D. in 38 patients

Immunity to Cholera

- Mucosal
  - Gastric acid, motility, mucus
    - Decreased acid associated with increased susceptibility, i.e., lower inoculum required to produce disease
- Immune system
  - Natural infection → long lasting protection
    - Antibody against O antigen and toxin (less important)
    - Principally mucosal IgA

Clinical Manifestations of Cholera

- Incubation period – 1-3 days
- Nonspecific prodrome: abdominal discomfort, vomiting, loose stools
- Rapid onset
- PROFUSE WATERY DIARRHEA
- No fever
- Stool is clear, odorless, “rice water”
- Dehydration, hypotension, acidosis, death
Sick Man with Cholera

Laboratory Diagnosis of Cholera
- Gram stain early
- Curved gram-negative rods
- Culture
  - Blood agar, enteric media
  - Selective media: Thiosulfate-citrate-bile salts-sucrose agar (TCBS agar)
  - PCR (included in multiplex PCR at LUMC)
  - 12 Vibrio species in stool samples in 2018

Management
- REHYDRATION
- Antibiotics
Intravenous Rehydration Solution

<table>
<thead>
<tr>
<th>Ions</th>
<th>mEq/Liter</th>
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</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>133</td>
</tr>
<tr>
<td>Chloride</td>
<td>98</td>
</tr>
<tr>
<td>Potassium</td>
<td>13</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>48</td>
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</table>

Glucose Linked Na Absorption

Oral Rehydration Solution

<table>
<thead>
<tr>
<th>Solute</th>
<th>mEq/Liter</th>
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<tbody>
<tr>
<td>Glucose*</td>
<td>120</td>
</tr>
<tr>
<td>Sodium</td>
<td>100</td>
</tr>
<tr>
<td>Potassium</td>
<td>10</td>
</tr>
<tr>
<td>Chloride</td>
<td>70</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>40</td>
</tr>
</tbody>
</table>

*Glucose in mMol/Liter
WHO Emergency ORS

- 1 L safe water
  - Salt – ½ small spoon (3.5 grams)
  - Sugar – 4 big spoons (40 grams)
- Potassium – Bananas or green coconut water

Antimicrobial Treatment of Cholera

- Doxycycline in most countries
- Azithromycin for women and children

Prevention of Cholera

- Sanitation
  - Handwashing
  - Boiling and disinfection of water
  - Thorough cooking of fish and shellfish
- Vaccines
  - Single dose oral live attenuated vaccine – CVD 103-HgR (Vaxchora) licensed by FDA
    - Travelers to cholera-affected areas
    - This and other vaccines distributed during epidemics
Cholera: Key Points
- Curved, gram negative, water and food borne bacteria
- Senses environmental signals with control of virulence factor gene transcription
- Causes widespread epidemics of diarrhea with significant mortality
- Non invasive, adheres, AB toxin which activates adenylate cyclase enhancing Cl secretion and blocking Na absorption
- Afebrile, watery diarrhea with profound fluid loss
- Can rehydrate orally with glucose-containing electrolyte solutions
- Antibiotic (azithromycin, doxycline) treatment beneficial and new vaccines are effective

Medically Important Vibrios

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Transmission</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio cholerae O1 and O139</td>
<td>Coastal waters</td>
<td>Ingestion (bathing) contaminated water</td>
<td>Severe watery diarrhea</td>
</tr>
<tr>
<td>Vibrio cholerae non O1, non O139</td>
<td>Coastal waters</td>
<td>Contaminated seafood</td>
<td>Cholera-like or milder diarrhea</td>
</tr>
<tr>
<td>Vibrio parahemolyticus</td>
<td>Coastal waters</td>
<td>Raw or inadequately cooked seafood, Water on Cruise Ship</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Vibrio vulnificus, others</td>
<td>Sea water</td>
<td>Direct contact: Bathing, fishing, Ingestion of shellfish</td>
<td>Wound infection, Septicemia in persons with liver disease</td>
</tr>
</tbody>
</table>

Campylobacter

| Campylobacter Species Associated with Human Disease |
|---------------------------------|------------------|-----------------|
| Species                        | Common Reservoir | Human Disease   |
| Campylobacter jejuni           | Poultry, cattle, sheep | Gastroenteritis, Enterocolitis, Campylobacteriosis   |
| Campylobacter coli             | Pigs, poultry, sheep, birds | Gastroenteritis, extra-intestinal             |
| Campylobacter fetus           | Cattle, sheep    | Septicemia       |
| Campylobacter upsaliensis     | Dogs, cats       | Gastroenteritis, extra-intestinal including GBS |
Campylobacter: Morphology and Staining

- Gram negative rod
- Curved
- Motile
- Pairs
- Seagull appearance

Culture Characteristics of Campylobacter

- Microaerophilic
- Oxidase positive
- Biochemically inactive
- Grow better at 42°C than 37°C

Epidemiology: Campylobacter jejuni

- 4 – 30% of infectious diarrhea
- Reservoir – GI and GU tracts of animals: sheep, cattle, chickens
- Raw or poorly cooked poultry responsible for 50-70% of sporadic infections
  - Minnesota study found in 74% of chicken samples
- Unpasteurized milk
- Contaminated well or surface water
- Person-to-person spread unusual
- C. upsaliensis – contact with domestic dogs
Common Sources of Campylobacter

- 48% – Chicken consumption
- 9% – Travel to underdeveloped countries
- 8% – Drinking non-home well or surface water
- 6% – Exposure to animal with diarrhea
- 5% – Drinking raw milk
- 24% – Unknown

CDC Foodnet


- 118 persons from 18 states
- 11 from Illinois
- 92 – contact with puppies from Company A, a national pet store chain based in Ohio
- 9 – contact with puppies from other pet store companies
- 39/45 tested strains related by wgMLST
- 95% of puppies received antibiotics before exposure
- 18 strains tested were resistant to all antibiotics used to treat campylobacter enteritis

Foodborne Campylobacter Outbreaks by Food Category, 2010-2015
2018 BioFire GI Panel Results
LUMC January to November 2018.
CDC Foodnet Summary 2017.

Pathogenesis of *C. jejuni*

- Inoculum – 500-10^4
- Attach – fimbriae, other protein adhesins
- Flagella enhance virulence (motility, invasion)
- Toxins of unknown significance identified
- Invasion, intracellular replication postulated
- Linked to Guillain-Barré Syndrome – autoimmune inflammatory neuropathy
  - About 1 per 1000 infections
  - Campylobacter infection precedes up to 20-50% of GBS
  - Cross reaction: bacterial ganglioside-like lipooligosaccharide (LOS) structures and human peripheral nerve gangliosides
    - Triggers both demyelination and axonal degeneration
  - Associated with Reactive Arthritis
  - Natural immunity

Clinical Manifestations of Campylobacter

Timeline of a Typical Attack of Campylobacter Enteritis
Laboratory Diagnosis of Campylobacter

- Culture stool
  - Selective media
  - Incubate in microaerophilic conditions
  - Elevated temperature
- PCR is replacing culture

Treatment of Campylobacter

- Erythromycin or azithromycin shorten the course and prevent relapse
- Ciprofloxacin is alternative
  - Ciprofloxacin resistance is increasing
- Treat for five days
- Not recommended for uncomplicated infection

Campylobacter: Key Points

- Curved gram negative rod, microaerophilic, oxidase positive, grows at higher temperatures (42°C)
- Common cause of invasive diarrhea from food (poultry), water and milk
- Three day incubation followed by fever, ABDOMINAL PAIN, with blood and pus in stool
- Relapse may occur
- Associated with Guillain-Barré Syndrome and Reactive Arthritis
- Treatment with erythromycin, azithromycin or ciprofloxacin
  - Ciprofloxacin resistance is increasing
  - Treatment for severe or immunocompromised only
Campylobacter fetus

- Rare cause of septicemia
- Localized infection may be present

Helicobacter pylori

- Colonizes, persists and causes inflammation in gastric mucosa
- Linked to upper GI diseases
  - Gastritis
  - Gastric and duodenal ulcer
    - Except that associated with NSAID's
  - Gastric carcinoma
  - Gastric mucosa-associated lymphoma (MALT)

Helicobacter: Morphology and Culture

- Curved, Gram-negative rods
  - Motile with corkscrew motility
- Culture
  - Microaerophilic
  - Oxidase positive
  - UREASE POSITIVE
  - Non-oxidative, non-fermentative
Epidemiology of Helicobacter

- Ingestion → persistent colonization → inflammation
- Acquired early in life
  - Developing countries - Seroprevalence 70-90%
  - In US Seroprevalence <40%
- Person-to-person predominates in developed world
- Linked to gastric and duodenal ulcer, gastritis, gastric cancer and gastric lymphoma

Seroepidemiology of *H. pylori*

![Graphs showing seroprevalence over age](image)

*H. Pylori* – Pathogenesis

- Unique characteristics allow barrier penetration and persistence in hostile environment
  - Mucinase – penetrates gastric mucosa
  - Flagella
  - Adherence to gastric (not duodenal) mucosa
    - Form membrane attachment pedestals
    - BabA binds to fucosylated Lewis blood group antigen receptor expressed on intestinal mucosal cells
    - Other adhesins
  - Urease
    - Formation of ammonia and CO2 creates mini alkaline environment

Genetic variation – 32 related OMPs include adhesins alter antigenic structure by several mechanisms
**H. Pylori** Vacuolating Cytotoxin

- Exotoxin inserts into cell membrane
  - Forms pore releasing nutrients
- Targets mitochondrial membrane
  - Releases cytochrome c
  - Induces apoptosis

**H. Pylori** cag Pathogenicity Island

- 30 genes that includes a type IV secretory system that inserts CagA and CagE into cell
  - Cag A, CagE and other cag proteins multiple functions
  - promote cytokine production, especially IL-8 that activates and attracts neutrophils

**Clinical Manifestations**

- Gastritis
  - Fullness, epigastric pain, nausea, vomiting, bleeding
- Gastric and duodenal ulcer
  - Pain and bleeding
- Gastric Cancer
  - Studies demonstrate 2 to 6-fold increase compared to uninfected populations
- Gastric mucosa-associated B-cell lymphoma
  - Pain or discomfort, loss of appetite, weight loss, bleeding
  - Treat Helicobacter→most early lesions regress
- Associated with immune thrombocytopenia (ITP)
H. Pylori – Diagnosis

- Noninvasive testing
  - Serology – before treatment
  - Breath test (measures ammonia)
  - Stool antigen test
  - PCR expensive and not widely used
- Invasive testing
  - Urease test – measures urease activity in gastric biopsy material
  - Histology
  - Culture – difficult
  - Required for antibiotic sensitivity testing

Treatment of H. pylori

- Combination of proton pump inhibitor and two antibiotics (amoxicillin, clarithromycin, metronidazole, tetracycline) for 10-14 days
  - Recent studies favor ten days of sequential treatment with two regimens each for five days
- Bismuth salts, doxycycline, metronidazole for 14 days is cheap and effective
Helicobacter: Key Points

- Curved gram negative rod, microaerophilic, oxidase positive, UREASE positive
- Persistent colonization of gastric mucosa causing inflammation
  - Well-orchestrated mechanism in which bacterial proteins induce host cytokine production
- Common - occurring at younger age in less developed countries
- Causes gastritis, gastric and duodenal ulcer, mucosa-associated B-cell lymphoma (MALT), gastric cancer and linked to ITP
- Diagnosed with invasive (biopsy) and non invasive (breath, stool antigen and antibody) tests
- Complex multidrug treatment regimens with antibiotics and PPIs