Gram Negative and Gram Positive Anaerobes

- Objectives and Key Concepts
  - Know which anaerobic species cause which infections
  - Identify key virulence factors important in pathogenesis of anaerobic bacteria
  - Know which anaerobic pathogens cause infection as single agents vs those that are typically part of a mixed infection
  - Know key microbiological features of anaerobic bacteria
  - Know key signs and symptoms of infections caused by various Clostridia species
  - Know which antibiotics that have good/excellent activity against anaerobes

Medically Important Anaerobes

- Gram positive rods
  - Clostridium spp.
  - Eubacterium
  - Lactobacillus
  - Actinomyces
  - P. acnes
- Gram positive cocci
  - Peptostreptococci
- Gram negative rods
  - Bacteroides fragilis group
  - Prevotella spp
  - Porphyromonas spp
  - Fusobacterium spp

Gram Positive Anaerobes (spore forming)

- Clostridium perfringens (single or mixed)
- Clostridium tetani (single)
- Clostridium botulinum (single)
- Clostridium difficile (single)
Clostridium perfringens

- Gas gangrene – toxin
- Intraabdominal infections- vegetative and toxin
- Food poisoning- toxin

Clostridium perfringens

- Structure:
  - Gram positive
  - Non-motile
  - Encapsulated
  - Forms spores
  - Double zone hemolysis

“Box Car” appearance of Clostridium spp
Gas Gangrene

• Pathogenesis
  – Trauma with devitalized tissue/muscle
  – Spores of C. perfringens, C. novyi, C. septicum, C. ramosum germinate quickly
  – Extremities, endometrium, abdominal wall
• Typing A-E – based upon combination of toxins produced by organism

Gas gangrene

• C. perfringens: Type A most common cause of human disease in U.S.
• Effects of toxins
  – α toxin -lecithinase (phospholipase C), lysis of inflammatory cells, tissue destruction (muscle)
  – β-toxin- enteritis necroticans (pig bel)
  – i – necrosis and vascular permeability
  – ε toxin- Systemic, vascular permeability
  – Θ toxin -Cardiotoxic
Gas Gangrene

• Clinical Disease
  – Rapid onset (6 - 72 hr)
  – necrosis of muscle (myonecrosis), skin
  – tense edema
  – bullae formation
  – gas formation (fermentation)
  – Systemic
    • shock
    • hyper or hypothermia

Gas gangrene

• Diagnosis
  – Clinical
    • suspicion
    • Gram stain
    • culture

• Treatment
  – debridement, debridement, debridement
  – antibiotics (penicillin, B-lactam/inhibitor)
    • clindamycin
**Clostridium perfringens**

- Food poisoning
  - Heat resistant spores survive
    - gravy, soups
  - enterotoxin
    - produced following germination of large numbers of organisms
- Clinical
  - 8 - 24 hr after ingestion
  - nausea, abdominal pain, diarrhea

**C. perfringens** (food poisoning)

- Diagnosis
  - clinical
  - culture (not needed)

- Treatment
  - self limited disease
  - supportive

**Clostridium tetani**

- Entry and Spread
  - puncture wounds
  - burns
  - umbilicus
  - local germination without necrosis
- Tetanospasmin
  - neurotoxin (plasmid borne)
    - blocks postsynaptic inhibition spinal motor reflexes
      - (GABA)
    - Spasmodic contractions
**Clostridium tetani**

- **Clinical**
  - Generalized
    - *trismus* (lockjaw)
    - *risus sardonicus* (increased tone orbicularis oris)
    - opisthotonus (arm flexion, leg extension)
    - Respiratory (obstruction, diaphragm)
  - Toxin
    - intraaxonal, transports cephalid over 2 weeks

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**CLOSIDIUM TETANI GRAM STAIN**

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**OPISTHOTONUS**
OPISTHOTONUS

Tetanus

- Localized
  - rigidity of muscles at site of sporulation
  - usually precedes generalized

- Cephalic
  - cranial nerve musculature
    - facial, ocular

- Neonatal
  - failure of aseptic technique

Risu Sardonicus
Tetanus

- Diagnosis
  - Clinical

- Treatment
  - Human tetanus immunoglobulin
  - Sedation
  - Control of spasms
  - Supportive (airway)

Tetanus

- Prophylaxis
  - 3 doses DTaP
  - Revaccination every 10 years (Tdap/Td)

- Prevention
  - Passive immunization
    - HTIG for appropriate wound
    - No previous vaccination
    - > 5 years

Clostridium botulinum

- Ubiquitous organism (A-G serotypes, A, B, E, F associated with human disease)
  - Spores heat resistant (100°C x hours)
  - Home canning fruits/vegetables, fish
  - Rarely commercial sources

- Entry
  - Preformed toxin from contaminated food
  - Spores in honey (infants)
  - 12 - 36 hrs after ingestion
**Clostridium botulinum**

- Toxin (bacteriophage borne)
  - large single polypeptide (150-165 Da)
  - cleaved by bacterial protease
  - most potent toxin in nature
  - blocks acetylcholine
  - no transmitter release, paralysis
  - synapse permanently damaged
  - can travel axons
  - heat labile

**Botulism**

- Clinical
  - GI (nausea, dry mouth, diarrhea)
  - descending paralysis (flaccid)
    - cranial (III, IV, VI)
    - symmetric neurologic effects
    - afebrile
    - patient conscious
    - heart rate normal
    - no sensory deficits
  - Wound botulism

**Diagnosis**

- Clinical (DDx myasthenia gravis, Eaton Lambert, tick paralysis, Guillain-Barre
- toxin assays (serum, stool, food)

**Prevention/Treatment**

- avoid contaminated food
- heating of food (boiling)
- antitoxin (equine serum to A,B,E)
- supportive
Clostridium difficile

- Major hospital acquired pathogen
  - Rare normal flora
  - spores acquired in hospital
  - antibiotic therapy usually precedes disease
  - Toxin A - enterotoxin, inflammatory response, major toxin
  - Toxin B - cytotoxic

Diffuse Hemorrhagic colitis and pseudomembrane formation

PSEUDOMEMBRANOUS COLITIS
C. difficile

- Clinical
  - Pseudomembranous colitis
  - Diarrhea (watery, severe, bloody)
  - Abdominal pain
  - Leukocytosis
  - Fever
  - Toxic megacolon

- BI (Nap1) Strain
  - Increased Toxin A production
  - Higher mortality, especially elderly
  - Up to 50% of isolates

C. difficile

- Diagnosis
  - PCR - New standard - amplifies toxin region
    - 90+% sensitivity, 98% specificity
  - Detection of toxin A in stool
    - ELISA
      - 85 - 90% sensitivity (single assay)
      - 95% sensitive (two assays)
  - Culture
    - Not routinely recommended
  - Sigmoidoscopy/colonoscopy

C. difficile

- Treatment
  - Stop antibiotics if possible
  - Metronidazole - mild/moderate (oral preferred)
  - Vancomycin (oral only)
  - Fidaxomicin - primary and relapse
  - Colon resection
  - Lactobacillus
  - Fecal Transplant (FMT)
**Clostridium septicum (single)**
- Nontraumatic myonecrosis
- Bacteremia- likely result of break in integrity of bowel mucosa
  - High association with colon cancer, leukemia
  - Fulminant disease with high mortality
- Clostridium sordelli (single)
  - Abortion (11%), pregnancy (18%), IVDA (22%), other (40%)
  - Profound leukocytosis
  - 50-100% mortality

**Other Anaerobic Gram positive Rods**
- Actinomyces- non spore forming (single or mixed)
  - Filamentous hyphae
  - Sulfur granules (yellow sand colonies)
  - Non-acid fast
- Clinical
  - Slow growing, suppurative
  - Typically oral associated
  - Cervicofacial, thoracic, pelvic or intraabdominal
- Diagnosis
  - Culture, but slow growing
- Treatment
  - Penicillin best
  - Clindamycin or erythromycin

**Propionobacterium acnes (single)**
- Slow growing anaerobe
  - Contaminant in blood cultures
  - Cause of infection:
    - Prosthetic devices or hardware
    - Opportunistic infections
    - Acne
  - Treatment – if indicated
    - Penicillin as well as many other agents except metronidazole!!
Anaerobic Gram positive cocci

- Peptostreptococcus (single or mixed)
  - Normal flora of mouth, GI tract, pelvis
  - Contiguous infections (usually mixed)
    - Intra-abdominal, endometritis, pulmonary
  - Brain abscess

- Diagnosis – culture
- Treatment – debridement and penicillin, metronidazole, cabapenems, clindamycin

Gram Negative Anaerobes

- B. fragilis group (mixed)
  - Non-spore forming, non-motile
  - Requires enriched media to grow
  - Normal inhabitant of GI tract (colon)
  - Provide protection from invasion (?)

Bacteroides fragilis group

- B. fragilis
- B. thetaiotaomicron
- P. distasonis
- B. ovatus
- B. caccae
- B. uniformis
- B. vulgatus
- B. eggerthiae
- B. merdae
- B. stercoris
**B. fragilis group Pathogenesis**

- Hallmark is abscess formation
  - Almost always mixed infections (aerobes and anaerobes)

- Abscess:
  - Inflammatory response to infection
  - Encapsulated “pus”
    - PMN’s
    - Fibrin, fibrinogen
    - Debris, necrotic tissue

**B. fragilis group**

- Virulence factors
  - Polysaccharide capsule
    - Adherence to peritoneal cavity
    - Resists phagocytosis
  - Resistance to T cell and humoral immunity
  - Oxygen tolerance
    - Super oxide dismutase
    - Catalase
  - Toxins: Defective endotoxin – not associated with sepsis

**B. fragilis group**

- Most commonly recovered from clinical infections
  - Intraabdominal (3/2 ratio anaerobe/aerobe)
  - Female genital tract (3/1 ratio anaerobe/aerobe)
  - Aspiration pneumonia (mixed)
  - Empyema (mixed)
  - Otitis media ?
  - Brain abscess (mixed)
  - Skin and Soft Tissue infections (3/1 ratio)
Peritonitis

Pathogens from 900 Intraabdominal Infections

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Isolates</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>483</td>
<td>38</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>124</td>
<td>10</td>
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<tr>
<td><em>Proteus sp.</em></td>
<td>141</td>
<td>11</td>
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<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>60</td>
<td>5</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<td><em>Staphylococcus</em></td>
<td>160</td>
<td>13</td>
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<tr>
<td>Other <em>streptococci</em></td>
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<td>1</td>
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<tr>
<td>Other aerobes</td>
<td>75</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,029</td>
<td>100</td>
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</table>

*Anaerobes*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>%</th>
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<tbody>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>329</td>
</tr>
<tr>
<td>Other <em>Bacteroides</em> species</td>
<td>318</td>
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<tr>
<td><em>Fusobacteria</em></td>
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<tr>
<td><em>Peptostreptococci</em></td>
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<td><em>Clostridia</em></td>
<td>609</td>
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<tr>
<td><em>Prevotella</em></td>
<td>47</td>
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<tr>
<td>Other</td>
<td>133</td>
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<tr>
<td><strong>Total</strong></td>
<td>1,378</td>
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Fournier Gangrene

Liver Abscess

Liver Abscess: Microbiology

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<thead>
<tr>
<th>BACTERIA</th>
<th>NUMBER OF LIVER ISOLATES</th>
<th>NUMBER OF BLOOD ISOLATES</th>
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<tr>
<td>Anaerobes</td>
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<tr>
<td>Peptostreptococci</td>
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<td>Microaerophilic streptococci</td>
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<td>Fusobacteria</td>
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<td>Bacteroides angilis</td>
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<tr>
<td>Other Bacteroides sp.</td>
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<td>Aerobes</td>
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<tr>
<td>Streptococci</td>
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<td>Escherichia coli</td>
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<tr>
<td>Proteus</td>
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</tbody>
</table>
Organisms Isolated from Diabetic Foot Infections

**Prevotella spp (mixed)**

- Residents of mouth, GI tract, and Pelvis
  - Gram negative strict anaerobes
  - Require enriched media (blood containing)
  - Non-motile
  - Non-encapsulated
  - Virulence – unknown
  - Pathogenesis – abscess formation

**Prevotella spp**

- Female genital tract infections
  - *P. bivia, P. disiens*

- Oral, pleuropulmonary
  - *P. oris, P. buccae, P. oralis*

- Oral cavity, urogenital, GI tract
  - *P. melaninogenica, P. corporis, P. denticola, P. intermedia, P. loescheii, P. nigrescens*
Other Gram negative Anaerobes
of Clinical Importance

- *Porphyromonas* (mixed or single)
  - oral, periodontal infections

- *Fusobacterium* (mixed or single)
  - *F. nucleatum*
    - aspiration pneumonia, lung abscess, empyema, chronic otitis media, sinusitis, brain abscess
    - liver abscess
  - *F. necrophorum*
    - Lemierre’s syndrome post anginal sepsis
    - widespread metastatic infection
    - Highly virulent – potent endotoxin
Antibiotic Therapy for Anaerobes

Non- C. difficile

- **Bacteroides**
  - Most Active:
    - Metronidazole (100%),
    - Ampicillin/sulbactam (96%),
    - Piperacillin/tazobactam (99%),
    - Carbapenems (99.5%),
    - Chloramphenicol (100%)
  - Formerly Active: Cefoxitin (75-90%), clindamycin (60%), piperacillin (60%)

- **Fusobacterium**
  - Most Active: metronidazole, amp/sulb, pip/tazo, clindamycin, cefoxitin
  - Active: piperacillin (?), penicillin (>80%)

- **Peptostreptococcus**
  - Most Active: Penicillin, clindamycin, amp/sulb, pip/tazo, metronidazole (some resistant strains)