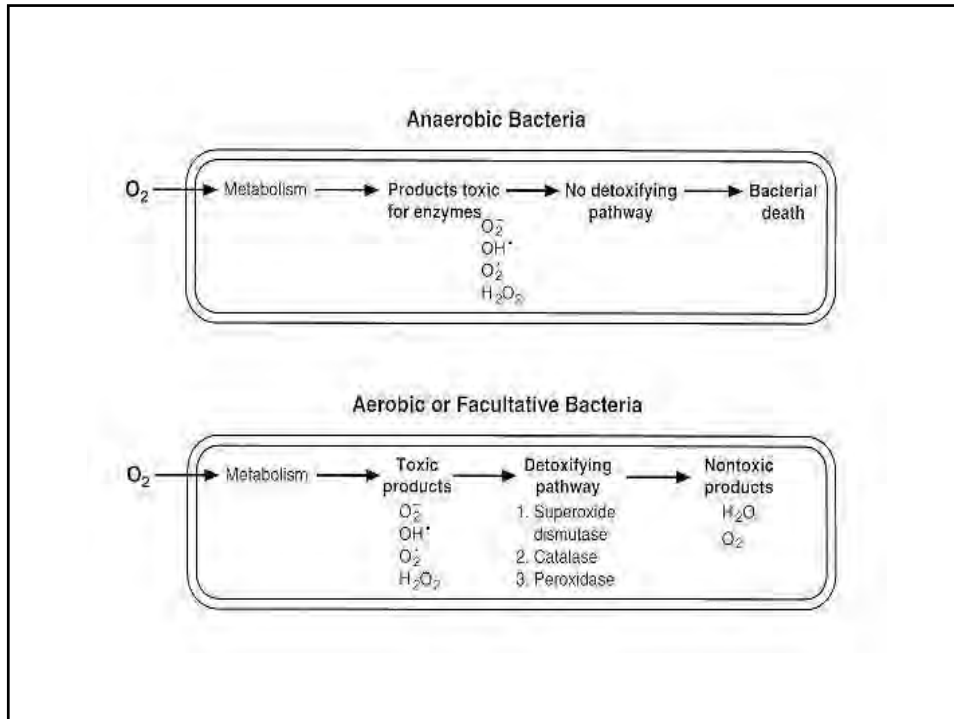


Anaerobes

Michael Yin, MD MS

Definitions

- **Anaerobes**
 - Bacteria that require anaerobic conditions to initiate and sustain growth
 - Ability to live in oxygen environment (detoxify superoxide ion)
 - Ability to utilize oxygen for energy instead of fermentation or anaerobic respiration
- **Strict (obligate) anaerobe**
 - Unable to grow if > than 0.5% oxygen
- **Moderate anaerobes**
 - Capable of growing between 2-8% oxygen
- **Microaerophilic bacteria**
 - Grows in presence of oxygen, but better in anaerobic conditions
- **Facultative bacteria (facultative anaerobes)**
 - Grows both in presence and absence of oxygen



Classification of Medically Important Anaerobes

- Gram positive cocci
 - Peptostreptococcus
- Gram negative cocci
 - Veillonella
- Gram positive bacilli
 - *Clostridium perfringens, tetani, botulinum, difficile*
 - Propionibacterium
 - Actinomyces
 - Lactobacillus
 - Mobiluncus
- Gram negative bacilli
 - *Bacteroides fragilis, thetaiotaomicron*
 - Fusobacterium
 - Prevotella
 - Porphyromonas

Epidemiology

- Endogenous infections
 - Indigenous microflora
 - Skin: Propionibacterium, Peptostreptococcus
 - Upper respiratory: Propionibacterium
 - Mouth: Fusobacterium, Actinomyces
 - Intestines: Clostridium, Bacteroides, Fusobacterium
 - Vagina: Lactobacillus
 - Flora can be profoundly modified to favor anaerobes
 - Medications: antibiotics, antacids, bowel motility agents
 - Surgery (blind loops)
 - Cancers
- Exogenous infections
 - Spore forming organisms in soil, water, sewage

Role of Anaerobes

- Prevent colonization & infection by pathogens
 - Bacterial interference through elaboration of toxic metabolites, low pH, depletion of nutrients
 - Interference with adhesion
- Contributes to host physiology
 - *Bacteroides fragilis* synthesizes vitamin K and deconjugates bile acids

Clinical features of anaerobic infections

- The source of infecting micro-organism is the endogenous flora of host
- Alterations of host's tissues provide suitable conditions for development of opportunist anaerobic infections
- Anaerobic infections are generally polymicrobial
- Abscess formation
- Exotoxin formation

Sites of anaerobic infections

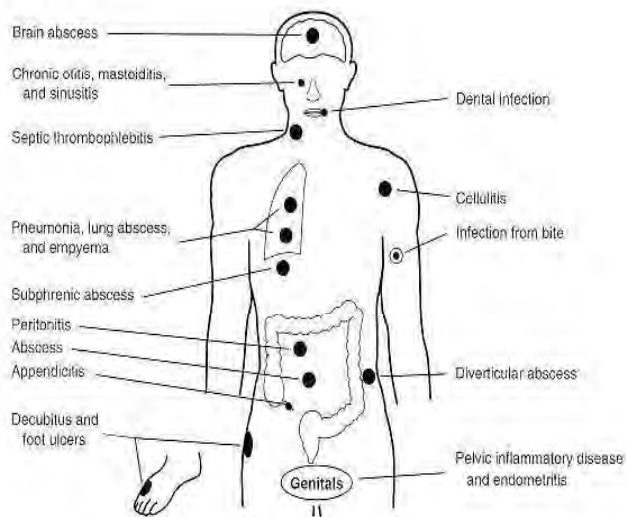


TABLE 20-2 Conditions Predisposing to Anaerobic Infection

General
Diabetes
Corticosteroids
Leukopenia
Hypogammaglobulinemia
Immunosuppression
Cytotoxic drugs
Splenectomy
Collagen disease
Decreased redox potential
Tissue anoxia
Tissue destruction
Aerobic infection
Foreign body
Calcium salts
Burns
Peripheral vascular insufficiency
Specific clinical situations
Cancer
Colon, uterus, lung
Leukemia
Gastrointestinal and female pelvic surgery
Gastrointestinal trauma
Human and animal bites
Aminoglycoside therapy

Virulence factors

- **Attachment and adhesion**
 - Polysaccharide capsules and pili
- **Invasion**
 - Aerotolerance
- **Establishment of infection**
 - Polysaccharide capsule (*B. fragilis*) resists opsonization and phagocytosis
 - Synergize with aerobes
 - Spore formation (Clostridium)
- **Tissue damage**
 - Elaboration of enzymes, toxins

Anaerobic cocci

- Epidemiology
 - Normal flora of skin, mouth, intestinal and genitourinary tracts
- Pathogenesis
 - Virulence factors not as well characterized
 - Opportunistic pathogens, often involved in polymicrobial infections
 - Brain abscesses, periodontal disease, pneumonias, skin and soft tissue infections, intra-abdominal infections
- Peptostreptococcus
 - *P. magnus*: chronic bone and joint infections, especially prosthetic joints
 - *P. prevotti* and *P. anaerobius*: female genital tract and intra-abdominal infections
- Veillonella
 - Normal oral flora; isolated from infected human bites

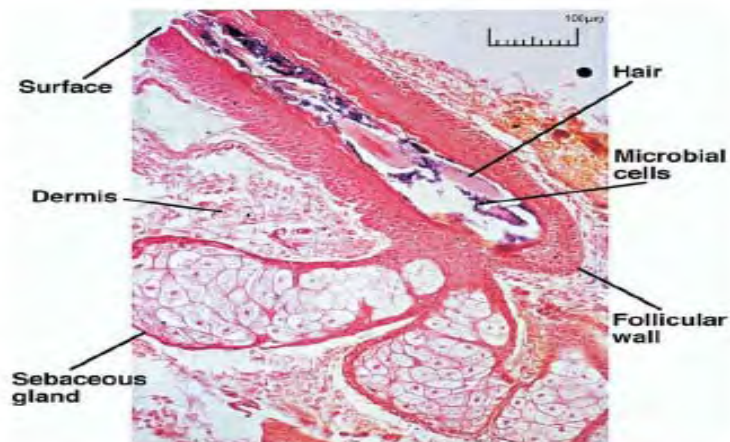
Anaerobic gram positive bacilli

- | | |
|----------------------|-------------------------|
| • No Spore Formation | • Spore Formation |
| – Propionibacterium | – Clostridium |
| • <i>P. acnes</i> | • <i>C. perfringens</i> |
| – Actinomyces | • <i>C. difficile</i> |
| • <i>A. israelii</i> | • <i>C. tetani</i> |
| – Lactobacillus | • <i>C. botulinum</i> |
| – Mobiluncus | |

Propionibacterium

- Produces propionic acid as major byproduct of fermentation
- Colonize skin, conjunctiva, external ear, oropharynx, female GU tract
- *P. acnes*
 - Acne
 - Resides in sebaceous follicles, releases LMW peptide, stimulates an inflammatory response
 - Opportunistic infections
 - Prosthetic devices (heart valves, ventricular shunts)

Pilosebaceous follicle



Actinomyces

- Facultative or strict anaerobe
- Colonize upper respiratory tract, GI, female GU tract
- Actinomycosis
 - Endogenous disease, no person-person spread
 - Low virulence; development of disease when normal mucosal barriers are disrupted (dental procedure)
 - Diagnosis made by examination of infected fluid:
 - Macroscopic colonies of organisms resembling grains of sand (sulfur granules)
 - Culture

Actinomycosis

- Cervicofacial Actinomycosis
 - Poor oral hygiene, oral trauma, invasive dental procedure
 - Chronic granulomatous lesions that become suppurative and form sinus tracts
 - Slowly evolving, painless process
 - Treatment: surgical debridement and prolonged penicillin



Lactobacillus

- Facultative or strict anaerobes
- Colonize GI and GU tract
 - Vagina heavily colonized ($10^5/\text{ml}$) by *Lactobacillus crispatus* & *jensonii*
 - Certain strains produces H_2O_2 which is bactericidal to *Gardnerella vaginalis*
- Clinical disease
 - Transient bacteremia from GU source
 - Bacteremia in immunocompromized host
 - Endocarditis

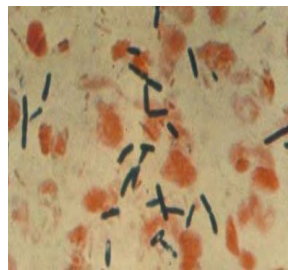
Case 1

- 12 year old boy with Acute Myelogenous Leukemia (AML) diagnosed 2 mo. ago
- Pancytopenia after receiving chemotherapy
- Presented with painful ecchymotic areas on legs that rapidly progressed with marked swelling and pain over several hours
 - Afebrile
 - Crepitus in both legs
 - Rapid progression to shock



Case 1

- Needle aspirate of ecchymotic area revealed gram-positive bacilli
- Blood cultures grew *Clostridium perfringens*

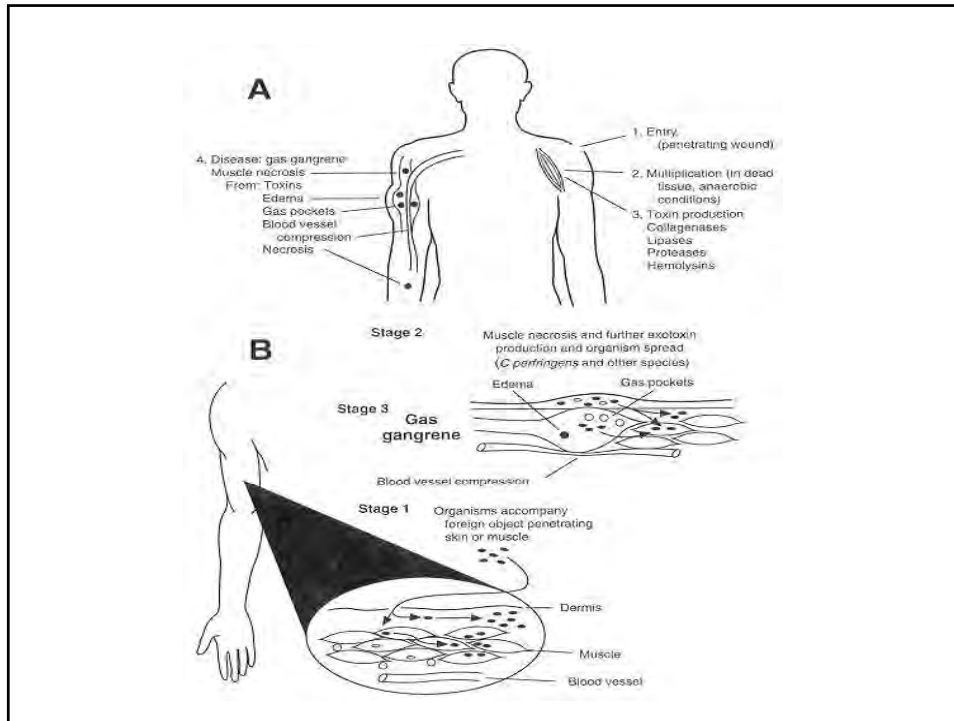


Clostridium

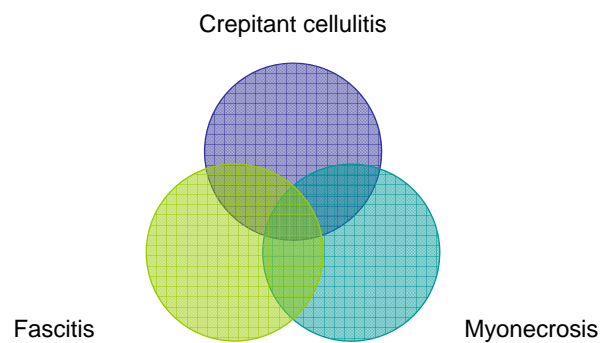
- Epidemiology
 - Ubiquitous
 - Present in soil, water, sewage
 - Normal flora in GI tracts of animals and humans
- Pathogenesis
 - Spore formation
 - resistant to heat, dessication, and disinfectants
 - can survive for years in adverse environments
 - Rapid growth in oxygen deprived, nutritionally enriched environment
 - Toxin elaboration (histolytic toxins, enterotoxins, neurotoxins)

Clostridium perfringens

- Epidemiology
 - GI tract of humans and animals
 - Type A responsible for most human infections, is widely distributed in soil and water contaminated with feces
 - Type B-E do not survive in soil but colonize the intestinal tracts of animals and occasionally humans
- Pathogenesis
 - **α -toxin**: lecithinase (phospholipase C) that lyses erythrocytes, platelets and endothelial cells resulting in increased vascular permeability and hemolysis
 - **β -toxin**: necrotizing activity
 - **Enterotoxin**: binds to brush borders and disrupts small intestinal transport resulting in increased membrane permeability
- Clinical manifestations
 - Self-limited gastroenteritis
 - Soft tissue infections: cellulitis, fascitis or myonecrosis (gas gangrene)



Clostridial soft tissue infections



Myonecrosis



Clostridial myonecrosis

- Clinical course
 - Symptoms begin 1-4 days after inoculation and progresses rapidly to extensive muscle necrosis and shock
 - Local area with marked pain, swelling, serosanguinous discharge, bullae, slight crepitation
 - May be associated with increased CPK
- Treatment
 - Surgical debridement
 - Antibiotics
 - Hyperbaric oxygen

Case 2

- 80 year old woman who was treated for a pneumonia with a cephalosporin
 - Well upon discharge from hospital
 - 10 days later develops multiple, watery loose stools and abdominal cramps
 - Fever, bloody stools, worsened abdominal pain

Case 2

- Leukocytosis with 80% neutrophils
- Fecal leukocytes
- Stool culture neg. for salmonella, shigella campylobacter, Yersinia spp
- Colonoscopy
 - White plaques of fibrin, mucous and inflammatory cells



Clostridium difficile

- Epidemiology
 - Endogenous infection
 - Colonizes GI tract in 5% healthy individuals
 - Antibiotic exposure associated with overgrowth of *C. difficile*
 - Cephalosporins, clindamycin, ampicillin/amoxicillin
 - Other contributing factors: agents altering GI motility, surgery, age, underlying illness
 - Exogenous infection
 - Spores detected in hospital rooms of infected patients
- Pathogenesis
 - **Enterotoxin (toxin A)**
 - produces chemotaxis, induces cytokine production and hypersecretion of fluid, development of hemorrhagic necrosis
 - **Cytotoxin (toxin B)**
 - Induces polymerization of actin with loss of cellular cytoskeleton

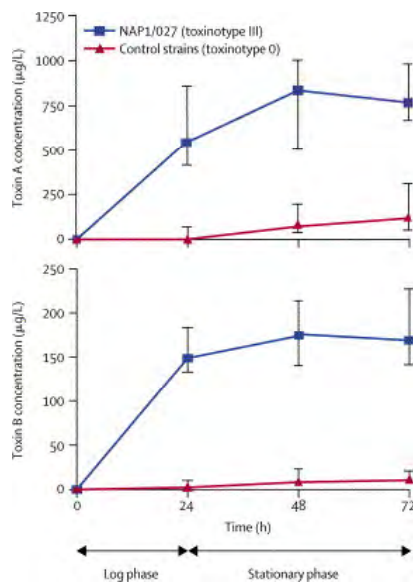
C. difficile colitis

- Clinical syndromes
 - Asymptomatic colonization
 - Antibiotic-associated diarrhea
 - Pseudomembranous colitis
- Diagnosis
 - Isolation of toxin
 - Culture
- Treatment
 - Discontinue antibiotics
 - Metronidazole or oral vancomycin
 - Pooled human IVIG for severe disease
 - Probiotics (*saccharomyces boulardii*)
 - New drugs (nitazoxanide, tolevamer)
 - Relapse in 20-30% (spores are resistant)

North American PFGE type 1 (NAP-1)

- Epidemiology:
 - Quebec 2003: 56.3/100,000; 18% severe, 14% died within 30 days
- Pathogenesis
 - Produces greater quantities of toxins A and B *in vitro*
 - Deletion in the *tcdC* gene (a putative negative regulator of toxin production)
 - Contains a binary toxin
 - Selected by fluoroquinolone use

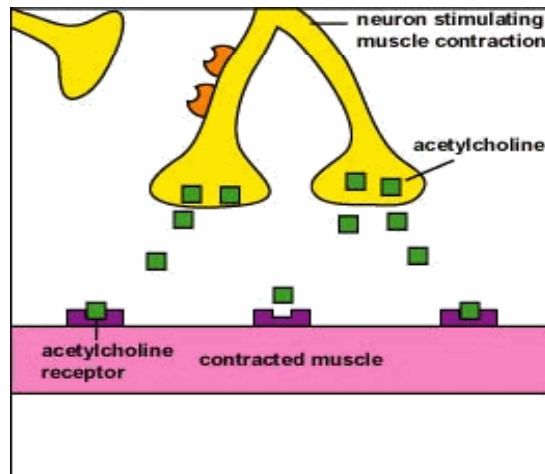
Wary et al, Lancet, 2005



Clostridium tetani

- Epidemiology
 - Spores found in most soils, GI tracts of animals
 - Disease in un-vaccinated or inadequately immunized
 - Disease does not induce immunity
- Pathogenesis
 - Spore inoculated into wound
 - **Tetanospasmin**
 - Heat-labile neurotoxin
 - Retrograde axonal transport to CNS
 - Blocks release of inhibitory neurotransmitters (eg. GABA) into synapses, allowing excitatory synapses to be unregulated. This results in muscle spasms
 - Binding is irreversible
 - Tetanolysin
 - Oxygen labile hemolysin, unclear clinical significance

C. tetani exotoxin



Tetanus

- Clinical Manifestations
 - Generalized
 - Involvement of bulbar and paraspinal muscles
 - Trismus (lock jaw), risus sardonicus, opisthotonos
 - Autonomic involvement
 - Sweating, hyperthermia, cardiac arrhythmias, labile blood pressure
 - Cephalic
 - Involvement of cranial nerves only
 - Localized
 - Involvement of muscles in primary area of injury
 - Neonatal
 - Generalized in neonates; infected umbilical stump

Risus sardonicus and Opisthotonos of Tetanus



Tetanus

- Treatment
 - Debridement of wound
 - Metronidazole
 - Tetanus immunoglobulin
 - Vaccination with tetanus toxoid
- Prevention
 - Vaccination with a series of 3 tetanus toxoid
 - Booster dose every 10 years

Case 3

- 6 month old infant girl, full-term, previously healthy
- Progressive fussiness, poor oral intake, weak cry for 4 days.
- Uninterested in feeding or playing.
- Exam:
 - Listless
 - Afebrile, stable vital signs
 - Sluggish pupils, decreased tone, no reflexes bilaterally



Case 3

- No ill contacts or recent travel, lives with parents on Staten Island
 - Construction in neighborhood
- Diet: Breast milk & some rice cereal only
- No fever, vomiting, diarrhea, rash, seizures

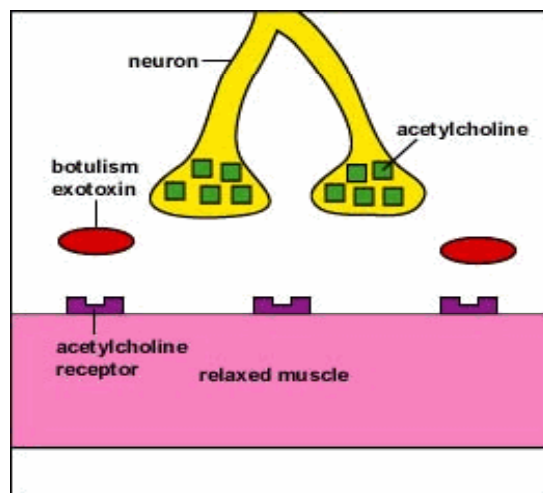
Case 3

- Serum, breast milk, stool sent to DOH for detection of Botulinum toxin
 - Stool POSTIVE for toxin type B
- Given Baby botulism immunoglobulin (Baby-BIG)
 - Regained movement of arm within a day
 - Began feeding in 4 days

Clostridium botulinum

- Epidemiology
 - Commonly isolated in soil and water
 - 20% soil samples
 - Human disease associated with botulinum toxin A, B, E, F
- Pathogenesis
 - Blocks neurotransmission at peripheral cholinergic synapses
 - Prevents release of acetylcholine, resulting in muscle relaxation
 - Recovery depends upon regeneration of nerve endings

C. Botulinum Exotoxin



Botulism

- Clinical Syndromes

- **Foodborne botulism**

- Associated with consumption of preformed toxin
 - Home-canned foods (toxin A, B)
 - Preserved fish (toxin E)
 - Onset of symptoms 1-2 days
 - Blurred vision, dilated pupils, dry mouth, constipation
 - Bilateral descending weakness of peripheral muscles; death related to respiratory failure

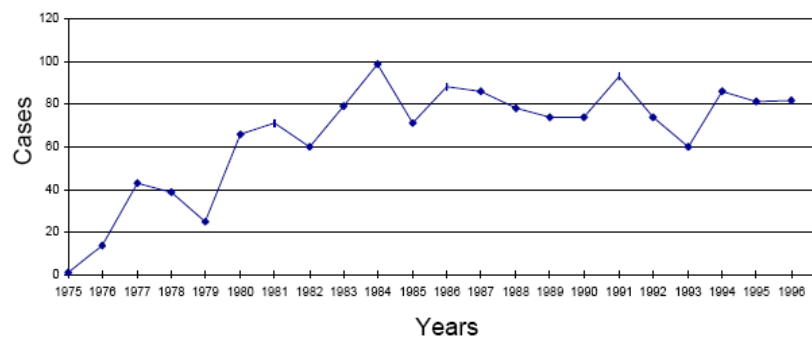
- **Infant botulism**

- Consumption of foods contaminated with botulinum spores
 - 6-10% of syrups or honeys
 - Disease associated with neurotoxin produced in vivo
 - Onset of symptoms in 3-10 days

- **Wound botulism** (skin popping)

- **Asymptomatic adult carriage**

Cases of Infant botulism 1976-1996



CDC, 1998

Botulism: diagnosis

- Clinical features:
 - Symmetric cranial nerve palsies (III, IV, VI, VII) causing 4Ds: diplopia, dysphonia, dysarthria, and dysphagia
 - Symmetric flaccid paralysis
 - Mentation remains intact
- Identification of toxin or organism in stool or serum
 - Mouse bioassay most sensitive
- Electromyography

Botulism: Treatment

- Treatment
 - Supportive care
 - Elimination of organism from GI tract
 - Gastric lavage
 - Metronidazole or penicillin
 - Botulinum Immunoglobulin (BIG): pooled plasma from adults immunized with pentavalent (ABCDE) botulinum toxoid
 - Trivalent equine Immunoglobulin (ABE)
- Prevention
 - Prevention of spore germination (Storage <4°C, high sugar content, acid PH)
 - Destruction of preformed toxin (20 min at 80°C)

Anaerobic gram negative bacilli

- Bacteroides
 - *B. fragilis*
 - *B. thetaiotaomicron*
- Fusobacterium
- Prevotella
- Porphyromonas

Anaerobic gram negative bacilli

- Epidemiology
 - Bacteroides and Prevotella are most prevalent organisms in human flora
 - **Oral cavity** (crypts of tonsils and tongue, dental plaques and gingival crevices)
 - Anaerobes become prominent after eruption of teeth
 - *Porphyromonas gingivalis* found in 37% of subjects, colonization concordance in families
 - Fusobacterium
 - **GI tract**
 - Anaerobes outnumber aerobes 1000:1
 - 10^{11} organisms per gram of fecal material
 - *Bacteroides* spp. (*vulgatus* and *thetaiotaomicron* most common)
 - **Vagina**

Anaerobic gram negative bacilli

- Clinical Diseases
 - Chronic sinus infections
 - Periodontal infections
 - Brain abscess
 - Intra-abdominal infection
 - Gynecological infection
 - Diabetic and decubitus ulcers

Case 4

- 37 year old woman with peri-umbilical pain, anorexia, and nausea
 - Given diagnosis of food poisoning in the ER and sent home
 - Develops sharp right lower abdominal pain and fever over next 4 days



Bacteroides

- Epidemiology
 - *B. fragilis* associated with 80% of intra-abd infx
 - Peritonitis, intraabdominal abscesses
 - Diabetic foot ulcers
- Pathogenesis
 - Polysaccharide capsule
 - Increases adhesion to peritoneal surfaces (along with fimbriae)
 - Protection against phagocytosis
 - Differs from LPS of aerobic GNR
 - Less fatty acids linked to Lipid A component
 - Less pyrogenic activity
 - Abscess Formation
 - Produces superoxide dismutase and catalase
 - Elaborate a variety of enzymes
 - Synergistic infections with aerobes

Abscess Formation

- Bacteroides Capsular Polysaccharide Complex (CPC)
 - 2 discrete polysaccharides (PS A & PS B) with oppositely charged structural groups
 - Injection of CPC into peritoneum of rat results in abscess formation
 - Chemical neutralization or removal of charged groups abrogated abscess induction
 - Vaccination with CPC results in protection against abscess formation
 - T cells important in abscess formation

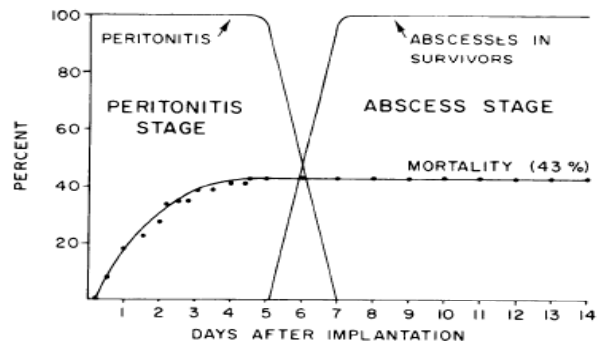


FIG. 1. Mortality and abscess formation in 106 male Wistar rats receiving inoculum obtained from meat-fed animals. Mortality is expressed as cumulative percent.

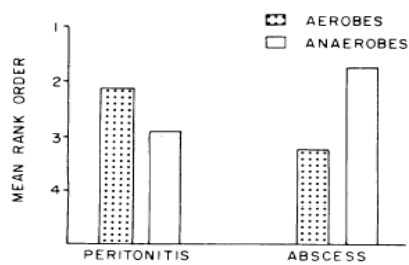


FIG. 2. Comparison of the mean rank order for the two major aerobic isolates (*E. coli* and enterococcus) and the two major anaerobic isolates (*B. fragilis* and *Fusobacterium*) in peritoneal exudates and abscesses. In peritonitis exudates, the rank order of the two aerobes was greater than that of the two anaerobes; in abscesses, anaerobes outranked aerobes. Factorial analysis of variance showed that this difference in rank order was significant ($P < 0.001$).

Abscess Formation

- Initial phase
 - Introduction of bacteria and inflammatory exudates (esp. fibrin)
- Microbial persistence (localization)
 - Impaired bacterial clearance: fibrin deposition, platelet clumping
 - Impaired phagocytic function: fibrin, hemoglobin
 - Impaired neutrophil migration and killing: hypoxia, low PH
 - Complement depletion: necrotic debris
- Development of mature abscess
 - Central core of necrotic debris, dead cells, bacteria
 - Surrounded by neutrophils and macrophages
 - Peripheral ring of fibroblasts and smooth muscle cells within collagen capsule

Conclusion

- Anaerobic infections
 - Endogenous or exogenous
 - Alteration of host tissue
 - Break in anatomic barrier
 - Devitalized tissue
 - Polymicrobial
 - Synergy between anaerobes and facultative bacteria
 - Abscess formation
 - Exotoxin elaboration