Anaerobes and Mycobacteria

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Objectives

- Review basic biology of select pathogens
- Epidemiology
- Pathogenesis
- Clinical findings
- Diagnosis
- Treatment and prevention
Electron Transport Chain - Generation of ATP

Aerobic Respiration - terminal electron acceptor is oxygen

Anaerobic Respiration - terminal electron acceptor
- Sulfate
- Nitrate
- Fumarate
- Trimethylamine Oxide

OR Fermentation - anaerobic metabolism ATP generated by substrate phosphorylation

- Hydrogen Sulfide (H₂S)
- Nitrite or Nitrogen Gas (N₂)
- Succinate
- Trimethylamine
Facultative anaerobes can use oxygen to grow when it is available but can also grow without it.

Obligate aerobes organisms that grow only in the presence of oxygen.

Obligate anaerobes organisms that grow only in the absence of oxygen.

Microaerophiles microbes that need lower concentrations of oxygen than are present in air. e.g. *Borrelia buradorferi*, a species of spirochaete bacteria that causes Lyme disease in humans, and *Helicobacter pylori*, a species of proteobacteria that has been linked to peptic ulcers and some types of gastritis.
Facultative Anaerobic Gram-ve Rods

- The Enterics
  - *Salmonella typhi*
  - *Shigella*
  - *Vibrio Cholerae*
  - *Yersinia pestis*
  - *E. coli*
- The Vibrios
  - *Vibrio cholerae*
- The Pasteurellas
  - *Pateurella*
  - *Haemophilus*
  - *Zymomonas*

Colonies of the bioluminescent marine bacterium *Vibrio fischeri.*
## Anaerobic Bacteria of Clinical Importance

<table>
<thead>
<tr>
<th>Genera</th>
<th>Anatomic Site</th>
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<tbody>
<tr>
<td><strong>Bacilli (rod)</strong></td>
<td></td>
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<tr>
<td>Gram-negative</td>
<td></td>
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<tr>
<td><em>Bacteroides</em></td>
<td>Colon</td>
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<tr>
<td><em>Fusobacterium</em></td>
<td>Mouth, Colon</td>
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<tr>
<td><em>Tannerella</em></td>
<td>Mouth</td>
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<tr>
<td><em>Prevotella</em></td>
<td>Mouth</td>
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<tr>
<td><em>Porphyromonas</em></td>
<td>Mouth</td>
</tr>
<tr>
<td><strong>Gram-positive</strong></td>
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<tr>
<td><em>Actinomyces</em></td>
<td>Mouth</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>Mouth, Vagina</td>
</tr>
<tr>
<td><em>Propionibacterium</em></td>
<td>Skin</td>
</tr>
<tr>
<td><em>Eubacterium, Bifidobacterium</em></td>
<td>Mouth, Colon</td>
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<tr>
<td>and <em>Arachnia</em></td>
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<tr>
<td><em>Clostridium</em></td>
<td>Colon, also found in the soil</td>
</tr>
<tr>
<td><strong>Cocci (spheres)</strong></td>
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<tr>
<td>Gram-positive</td>
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<tr>
<td><em>Peptostreptococcus</em></td>
<td>Colon</td>
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<tr>
<td>Gram-negative</td>
<td></td>
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<tr>
<td><em>Veillonella</em></td>
<td>Mouth, Colon</td>
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</tbody>
</table>
Polymicrobial nature of anaerobic infections

Contamination of tissues by normal flora of the mucosa of
- Mouth
- Pharynx
- Gastrointestinal tract
- Genital tract

Infections
- 25% anaerobes alone
- 25% facultative anaerobes
- 50% anaerobes and facultative anaerobes
Diagnosis and Treatment of Anaerobic Infections

Clinical signs
- Necrotic tissue
- Foul smelling discharge (due to short-chain fatty acid products of anaerobic metabolism)
- Infection in proximity to a mucosal surface (anaerobes are part of the normal flora)
- Gas in tissues (production of CO$_2$ and H$_2$)
- Black discoloration of exudates (gas gangrene)
- Negative aerobic cultures

Antibiotics, clindamycin, metranidazole, but penicillin G remains the drug of choice for treatment of anaerobic infection that do not involve *Bacteroides*
**Culture of Anaerobic Bacteria**

- **Glove Box:** 80% nitrogen, 10% hydrogen, 10% carbon dioxide
- **Gas Pack:** hydrogen and carbon dioxide generator envelope is placed in the jar along with the culture plates. The generator is activated with water. Oxygen within the jar and the hydrogen that is generated are converted to water in the presence of the catalyst
Isolation and identification of Anaerobes

Anaerobic specimen collection

Anaerobic isolation procedure

Anaerobe identification

- Colony morphology
- Gram staining reaction
- Antibiotic sensitivity patterns
- Carbohydrate fermentation reactions
- Biochemical tests
- Gas chromatography of metabolic products
Peptostreptococcus spp.

- *Peptostreptococcus magnus*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevotii*, and *Peptostreptococcus micros*
- Anaerobic, Gram+ve cocci
- Normal flora, mouth, skin, gastrointestinal and urinary tract
- Virulence factors are unknown.
- Brain abscess, plueropulmonary infection, respiratory tract, dental, Intra-abdominal and wound infections
- Treatment, penicillin, surgical drainage
- This is an opportunist that is often involved in polymicrobial infections. Injury that produces an interruption of the capillary blood flow thus lowering the oxygen potential can predispose to infection by any of the obligate anaerobes.
Propionibacterium acnes

- Anaerobic, slow-growing, nonsporeforming, Gram+ve,
- Normal flora of the skin, colonizes follicles of sebaceous glands
- Metabolic products include propionic acid
- Acne- produce inflammatory response from release of bacterial enzymes and hydolytic enzymes released from phagocytic cells leading to rupture of follicle
- Treatment
  - Topical cleansing agent (benzoyl peroxide)
  - Antibiotic (e.g., erythromycin, clindamycin)
- Opportunistic infection plastic shunts and appliances
**Clostridium botulinum**

- Normally a soil microbe, but it can also grow and produce botulinum toxin in improperly preserved foods. The consumption of botulinum toxin results in botulism, which can result in death due to respiratory failure from flacid muscle paralysis.

- Characteristics include abdominal pain, vomiting, motor disturbances, and visual difficulties.

- Gram+ve spore-forming rod

- Produces potent neurotoxin

- Seven types (A, B, C, D, E, F and G) of botulism, based on the antigenic specificity of the toxin produced by each strain. Types A, B, E and F cause human botulism. They have neuro-, entero-, and hemotoxic properties, are immunogenic, and include the most potent poisons known. The most commonly used apparently blocks release of acetylcholine at cholinergic synapses.
Endospores are a subclass of spores that are very resistant to harsh conditions. They can survive high heat (>100°C), drying, radiation, and many damaging chemicals.

Endospores can germinate many years after formation, even millions of years later.

Resistant to ultraviolet and gamma radiation, dessication, lysosyme, temperature, starvation, and chemical disinfectants. Endospores are commonly found in soil and water, where they may survive for long periods of time.

**Function.** ensure the survival of a bacterium through periods of environmental stress.

Variations in endospore morphology. 
(1, 4) Central endospore, (2, 3, 5) terminal endospore, (6) lateral endospore
Endospore

Fig. 8.1. Endospore
Botulism

The Centers for Disease Control and Prevention classify botulism into four types:

- **Foodborne** - consumption of foods containing the neurotoxin

- **Infant botulism** - affects infants under 12 months of age. Ingestion of *C. botulinum* spores which colonize and produce toxin in the intestinal tract of infants eg honey

- **Wound botulism** - rarest form. *C. botulinum* infects a wound and produces toxins which reach other parts of the body via the bloodstream.

- **Undetermined** - adult cases when a specific source is not identified

**Diagnosis** - demonstrate the presence of toxin in the serum or feces of the patient or in the food which the patient consumed. Currently, the most sensitive and widely used method for detecting toxin is the mouse neutralization test. This test takes 48 hours. Culturing of specimens takes 5-7 days.
• **Clinical symptoms.** Botulinum toxin causes flaccid paralysis by blocking motor nerve terminals at the myoneural junction. The flaccid paralysis progresses symmetrically downward, usually starting with the eyes and face, to the throat, chest and extremities. When the diaphragm and chest muscles become fully involved, respiration is inhibited and death from asphyxia results.

• **Treatment.** Foodborne botulism includes early administration of botulinal antitoxin (available from CDC) and intensive supportive care (including mechanical breathing assistance).

• **Outbreaks.** Anywhere from 20 to 50 outbreaks of botulism are reported annually in the United States. All people are susceptible to food-borne toxins, but food poisoning can be prevented by properly processing and preserving foods. Do not use canned foods that are bulging or have a foul odor.
**Clostridium difficile**

- Present as one of the normal bacteria in the gut
- Illness pseudomembranous colitis
  - when antibiotics disturb the normal gut flora
  - Resistant to most antibiotics
  - Treatment; Vancomycin and Metronidazole
- Spores can spread from person to person, bad hygiene
- Detection ELISA for toxin in stools
*Clostridium difficile* spores and vegetative cells are ingested

- Spores
- Vegetative cells

Most vegetative cells are killed in the stomach, but spores can survive the acid environment.

*C. difficile* multiplies in the colon.

Gut mucosa facilitates adherence to the colonic epithelium.

*C. difficile* spores germinate in the small bowel upon exposure to bile acids.

Flagellae facilitate *C. difficile* movement; a polysaccharide capsule discourages phagocytosis.
C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (1). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (2), opening of epithelial cell junctions (3) and epithelial cell apoptosis (4). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (5) and watery diarrhea.
Clostridium perfringens

- Anaerobic, Gram+ve sporeforming rod
- Widely distributed in the environment, intestines of humans, animals, soil, persistant
- Food poisoning- intense abdominal cramps 8-22 hrs after eating, over in 24 h
- Food infection, very rare to get symptoms from preformed toxin
- Gas gangrene
Clostridium tetani

- Anaerobic, Gram+ve sporeforming rod
- Soil, animal faeces and, occasionally, human faeces.
- Latent, but normally, incubation period is a few days to a few weeks. Spores deposited in dead (oxygen-free) tissue through a wound, burn, or ulcer.
- Neurotoxin. *tetanospasmin*, blocks the release of neurotransmitters from the presynaptic membranes of inhibitory nervesynapses.
- Clinical. Mild muscle contractions at the site of infection as the infection gradually spreads along nerve fibers to the spinal cord and brain stem. Trismus (lockjaw) ensues with continued rigidity and spasms of the extremities. Death occurs when spasms interfere with respiration.
- Treatment. If not sought early, often fatal. Antibiotics, delivery of antitoxin, and surgery
- Cases. Approximately 50 cases of tetanus (spastic paralysis) every year in the U.S. and one million worldwide
- Vaccine. Inactivated formaldehyde-treated toxin. Boosters are recommended every 7-10 years.
CDC Media Update:
XDR TB Public Health Investigation

FOR IMMEDIATE RELEASE
June 8, 2007

Contact: CDC Media Relations
(404) 639-3286

• The Centers for Disease Control and Prevention (CDC) provides the following update regarding its investigation and public health actions related to a patient with extensively drug-resistant tuberculosis (XDR TB). CDC is recommending that passengers and crew on two trans-Atlantic flights taken by the patient be notified of potential exposure to tuberculosis and evaluated for TB. On May 12, the patient flew from Atlanta to Paris on Air France flight #385/Delta Airlines flight #8517. On May 24, the patient flew from Prague, Czech Republic, to Montreal, Canada, on Czech Air flight #0104. There were 276 U.S. residents or citizens on the Air France/Delta flight and two on the Czech Air Flight (the patient and his wife).

• **Update: Notification of Passengers**  CDC communicated with most of the U.S. passengers who were on the patients flights. As of today, CDC officials have directly contacted 255 of the 274 U.S. passengers (93%) on the May 12 Air France/Delta flight from Atlanta to Paris.

• **Passenger Testing Recommendation**  As a reminder, CDC is recommending that passengers on the affected flights receive evaluation for signs and symptoms of TB, and a TB skin test or blood test for TB infection as soon as possible, and another TB test eight to 10 weeks after the flight. The first test will help determine whether passengers had pre-existing TB infection prior to the flight in question. The second test will help assess whether passengers may have become infected with TB while on the affected flight. An initial positive TB skin test doesn't necessarily mean that a person got TB infection on this flight.
Mycobacterium tuberculosis

- Gram+ve aerobic straight or slightly curved bacillus, surrounded by waxy coating formed by mycolic acid “acid fast bacteria”

- Transmission occurs by inhalation of droplet nuclei, aerosols caused by an infectious person coughing
  - Droplet nuclei contain 1-3 organisms, one organism may be enough to establish an infection
- The main site of infection is the lung (>80%), but any organ can be infected when dissemination occurs either by lymphatic drainage or haematological spread
- The principal lesion is the 'tubercle' (a granuloma)
- Intra-cellular pathogen, *M. tuberculosis* infects alveolar macrophages
  - The organism is phagocytosed by the macrophage and is found in membrane-bound particles known as 'phagosomes'
  - The infected phagosome does not mature, but remains in an state similar to the 'early endosome'
  - The phagosome does not acidify or fuse with lysosomes
  - The infected macrophage is unable to kill the organism

- TB is a chronic disease and an infected person can spread the infective organism, *M. tuberculosis*, to several contacts before being diagnosed
The Acid-Fast Stain

• Because of the waxy substance (mycolic acids) present on the cell wall, cells of species of *Mycobacterium* do not stain readily with ordinary dyes (Gram stain).

• Treatment with cold **carbol fuchsin** for several hours or at high temperatures for five minutes will dye the cells. Once the cells have been stained, subsequent treatment with a dilute hydrochloric acid solution or ethyl alcohol containing 3% HCl (acid-alcohol) will not decolorize them.

• Cells are thus termed acid-fast in that the cell will hold the stain *fast* in the presence of the acidic decolorizing agent.

• Microscopic examination of tissues or of sputum stained by the acid-fast staining procedure is an aid in the diagnosis of tuberculosis.
Clinical Symptoms of Tuberculosis

- many symptoms are due to overproduction of TNF
- severe weight loss
- night sweats
- chronic cough
- hemoptysis

Tuberculosis Latency

- Latency- a person may be infected without developing disease, infecting organisms remain viable
- Disease may be activated by malignancy, immune suppression, old age or chronic ill-health
- 10% life-long risk of developing disease (cf HIV/AIDS patients who have a 10% annual risk of developing disease)
Tuberculin Skin Test

- Tuberculin is a partially purified extract of *M. tuberculosis* proteins (PPD)
- PPD evokes a delayed hypersensitivity (DTH) response when injected into the skin—this forms the basis of the Tuberculin Skin Test
- Tuberculin skin testing can be used to identify individuals, especially children, with active tuberculosis
- It can be used to trace contacts of patients with active tuberculosis
- A positive tuberculin test may be an indication for INH prophylaxis
Persons at Higher Risk for Exposure to and Infection with *M. tuberculosis* (1)

- Close contacts
- Foreign-born persons from or areas with high TB incidence
- Residents and staff of high-risk congregate settings
- Health-care workers who serve high-risk clients
Persons at Higher Risk for Exposure to and Infection with *M. tuberculosis*

- HCWs unknowingly exposed to TB patient
- Low-income, medically underserved groups
- Locally defined high-risk groups
- Young persons exposed to high-risk adults
TB Patient Characteristics That Increase Risk for Infectiousness (1)

- Coughing
- Undergoing cough-inducing or aerosol-generating procedure
- Failing to cover cough
- Having cavitation on chest radiograph
Environmental Factors That Increase Risk for Transmission

- Exposure in small, enclosed spaces
- Inadequate ventilation
- Recirculating air containing infectious droplets
- Inadequate cleaning and disinfection of equipment
- Improper specimen-handling procedures
Risk for Health-care–Associated Transmission of *M. tuberculosis*

Risk varies by

- TB prevalence in health-care setting
- TB prevalence in community
- Patient population served
- Health-care worker occupational group
- Effectiveness of infection control measures
Mycobacterium lepre

Gram+ve aerobic rod, surrounded by waxy coating formed by mycolic acid unique to Mycobacterium. Never been grown in artificial culture but will grow on the footpads of mice and in armadillos, culture takes several weeks to mature. Transmission not fully understood, direct contact, air dispersion. Incubation period as long as 20 years or as short as 2 years. Hanson’s disease, Leprosy, skin lesions in early stages, leads to paralysis or loss of sensation in those areas, eventual loss of extremities, blindness. Treatment, Dapsone, MDT multidrug therapy for resistant strains. In 1997, there were approximately 1.2 million cases worldwide, with Africa and Asia reporting the highest numbers. Additionally, about 600,000 new cases are reported annually.

Dasypus novemcinctus, the nine-banded armadillo, the only known nonhuman natural host of the leprosy bacillus.