Anaerobes

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Recommended reading: Murray PR, editor: *Medical Microbiology*, ed 4, St Louis, 2002, Mosby, pages 334-358.

I. Introduction
The anaerobes are a heterogeneous group of bacteria that characteristically colonize the skin and mucosal surfaces. With the exception of the clostridial diseases in which specific exotoxins are responsible for clinical effects, three main features are common to most anaerobic infections: (1) the source of the infecting microorganism is the endogenous flora of the patient's own oropharyngeal, gastrointestinal, or genitourinary mucosa (2) alterations of the host's tissues provide suitable conditions for the development of opportunistic anaerobic infections (e.g., trauma, hypoxia) (3) anaerobic infections are generally polymicrobial, often involving mixtures of several anaerobic and aerobic species acting synergistically to cause damage.

II. Microbiology
Anaerobes are bacteria that require anaerobic conditions to initiate and sustain growth; however, oxygen tolerance (aerotolerance) is highly variable. For practical, operational purposes, an anaerobe can be defined as a bacterium that fails to grow on the surface of solid media in the presence of room air (10% carbon dioxide, 18% oxygen). **Strict anaerobes** are unable to grow in the presence of more than 0.5% oxygen, and **moderate anaerobes** are capable of growing at between 2% to 8% oxygen. Additionally, **facultative** bacteria can grow both in the presence and absence of air and **microaerophilic** bacteria grow poorly or not at all in air, but distinctly better in anaerobic conditions.

Anaerobes lack the cytochromes required to use oxygen as a terminal electron acceptor in energy-yielding reactions, and thus generate energy solely by fermentation. Some anaerobes will not grow unless the oxidation-reduction potential is extremely low because critical enzymes must be in the reduced state to be active (aerobic conditions create a metabolic block). Environments most favorable to anaerobic growth are often created by the presence of facultative organisms whose growth reduces oxygen and decreases the local oxidation-reduction potential These sites include the sebaceous glands of the skin, the gingival crevices of the gums, the lymphoidal tissue of the throat, and the lumina of the intestinal and urogenital tracts.

Table 1: Features of Pathogenic Anaerobes

Organism	Bacteriologic features	Exotoxins	Source	Disease
Gram-Positive Cocci				
Peptostreptococcus			Mouth, intestine	Oropharyngeal infections, brain abscess
Gram-Negative Cocci				
Veillonella			Intestine	Rare opportunist

Gram-Positive Bacilli				
Clostridium *perfringens*	Spores	α-toxin, θ-toxin, enterotoxin	Intestine, environment	Cellulitis, myonecrosis, enteritis
Clostridium *tetani*	Spores	Tetanospasmin	Environment	Tetanus
Clostridium *botulinum*	Spores	Botulinum	Environment	Botulism
Clostridium *difficile*	Spores	A enterotoxin, B cytotoxin	Intestine, environment	Pseudomembranous colitis
Propionibacterium			Skin	Acne, rare opportunist
Actinomyces			Upper respiratory, intestine	Actinomycosis
Lactobacillus			Mouth, intestines, genitourinary	Rare bacteremia
Gram-Negative Bacilli				
Bacteroides *fragilis*	Polysaccharide capsule	Enterotoxin	Intestine	Opportunist, abscess
Bacteroides species			Intestines	Opportunist, abscess
Fusobacterium			Mouth, intestines	Opportunist, abscess
Prevotella	Black pigment		Mouth, urogenital	Opportunist
Porphyromonas			Mouth, urogenital	Opportunist

III. Virulence Factors

Several key stages are common to most infective processes, although their relative significance differs with the type of infection. Virulence factors enable species to elicit these stages of infection: attachment to target cells (mucosal or epithelial); invasion into the tissue; multiplication at the site of infection and avoidance of elimination by host defense mechanisms; and mediation of tissue destruction.

Attachment and adhesion
The **polysaccharide capsule** of Bacteroides *fragilis* and Prevotella *melaninogenica* allows these organisms to adhere to peritoneal surfaces more effectively. The **Pili (fimbriae)** allow Bacteroides species and Porphyromonas *gingivalis* (commonly associated with periodontal disease in adults) to adhere to epithelial cells more effectively.

Invasion
Most anaerobes do not contain special virulence factors for invasion; therefore, infection depends upon **alteration in host tissue** such as trauma (gunshot, surgery), disease (diverticulosis) or isolated events (aspiration). Host factors such as malignancy or impaired blood supply increase the probability that dislodged flora eventually produce an infection. The anaerobes involved are normally found at the site adjacent to the infection: colonic flora are most frequently isolated in intra-abdominal abscesses; oropharyngeal

anaerobes are most commonly isolated in brain abscesses (extension across the cribriform plate to the temporal lobe) and lung abscesses (aspiration).

For anaerobes, the ability to survive brief exposures to oxygenated environments can also be viewed as a virulence factor. Most anaerobes lack the ability to produce **catalase** and **superoxide dismutase**, which are enzymes that inactivate hydrogen peroxide and superoxide free radicals (O_2^-). The presence of these enzymes allows the organism to have greater tolerance of oxygen exposure and increases its chance of survival during displacement. Bacterioides *fragilis,* and other pathogenic anaerobes, are far more likely to have the ability to produce catalase and superoxide dismutase.

Establishment of infection
The **polysaccharide capsule** of Bacteroides *fragilis* interferes with the host processes of opsonization and phagocytosis. The most distinguishing pathogenic feature of this Bacteroides *fragilis* is its ability to form an abscess. Experimentally, this capsule has been shown to produce abscesses even in the absence of living bacteria. This property is not found in capsular polysaccharaides of organisms such as Streptococcus *pneumoniae.* B. *fragilis* also produces a number of extracellular enzymes (collagenase, fibrinolysin, heparinase, hyaluronidase) that may also contribute to the formation of the abscess.

The clostridial species have the ability to survive adverse environmental conditions through **spore formation**. Spores are resistant to heat, desiccation, and disinfectants. They are able to survive for years in the environment and return to the vegetative form when placed in a favorable milieu.

The ability of bacteria to create and control a **reduced microenvironment**, often with the apparent help of other bacteria, is a feature that helps to establish anaerobic infections. The great majority of anaerobic infections are mixed infections, involving two or more anaerobes often in combination with facultative bacteria like Escherichia *coli.* These bacteria may synergize each other's growth by lowering the oxidation-reduction potential, providing growth factor, or inhibition host defenses under anaerobic conditions (oxygen-dependent leukocyte bactericidal functions).

Tissue damage
Clostridium can cause a wide spectrum of diseases ranging from self-limited gastroenteritis to myonecrosis to neuromuscular disease like tetanus and botulism depending upon the selective **elaboration of toxins**. Some toxins are enzymes that mediate the destruction of host tissue (α-toxin) and others block important metabolic processes of host cells (tetanospasmin, botulinum toxin). The following table details some of the important toxins elaborated by Clostridium.

Table 2. Toxins associated with Clostridium

Organism	Toxin	Biologic Activity
C. *perfringens*	α-toxin	Lecithinase (phospholipase C) that lyses erythrocytes, platelets and endothelial cells. This toxin increases vascular permeability, resulting in massive hemolysis and bleeding and tissue destruction (myonecrosis)

	β-toxin	Necrotizing activity, responsible for lesions in necrotizing enteritis
	θ-toxin	Heat and oxygen labile hemolysin; cytolytic
	Enterotoxin	Heat-labile, produced during phase transition from vegetative cells to spores and released when cells are lysed. Binds to the brush border membrane of the small intestine and disrupts transport and membrane permeability
C. *tetani*	Tetanolysin	Oxygen-labile hemolysin of uncertain clinical significance
	Tetanospasmin	Heat-labile neurotoxin produced during the stationary phase of growth, which is released when the cell is lysed. Tetanospasmin is a metalloproteinase that enzymatically degrades a protein required for docking of neurotransmitter vesicles on presynaptic membranes. Loss of this function blocks the release of neurotransmitters for inhibitory synapses causing excitatory synaptic activity to be unregulated. Toxin binding is irreversible.
C. *botulinum*	Botulinum	Blocks neurotransmission at peripheral cholinergic synapses by preventing release of the neurotransmitter acetylcholine. Binding is irreversible and recovery depends upon regeneration of nerve endings.
C. *difficile*	Toxin A (Enterotoxin)	Produces chemotaxis of PMN's, induces cytokine production, hypersecretion of fluid, and hemorrhagic necrosis
	toxin B (Cytotoxin)	Induces polymerization of actin with loss of cellular cytoskeleton.

IV. Clinical features of anaerobic infections

A. Intra-abdominal infection
Secondary peritonitis and intra-abdominal abscesses begin with the entry of gastrointestinal flora into the sterile peritoneal cavity, through a defect in the wall of the intestines (perforation) as a result of obstruction, infarction, direct trauma (gunshot wound, surgery) or inflammatory processes (inflammatory bowel disease, diverticulitis, appendicitis). Peritonitis is usually polymicrobial, and because anaerobes account for over 99% of the organisms found in normal colonic flora, most of the organisms recovered are anaerobes. Most intra-abdominal infections have a bi-phasic clinical presentation. Initially, there is generalized peritonitis characterized by fever, diffuse abdominal pain, nausea, vomiting, and a rigid abdomen, which may be followed by signs and symptoms of shock. The clinical presentation of the second stage, which is characterized by a formation of an intra-abdominal abscess, may be quite variable depending upon modification by antibiotics. Intra-abdominal abscesses are typically polymicrobial, and anaerobes are recovered in 60-70% of cultures. Bacteriodes species, especially B. *fragilis*, are the most common pathogen, followed by anaerobic cocci and Clostridia. Other frequently recovered pathogens from abscesses are E. *coli*, the Klebsiella/Enterobacter group, Proteus spp., P. *aeruginosa*, S. *aureus*, and enterococci. Successful treatment of an abscess usually requires drainage of the pus as well as antibiotic therapy. Effective management depends upon accurate localization of the abscess, discrimination between single and multiple abscesses, and early and adequate drainage. The best way to drain an abscess depends on the clinical situation. Incision, needle aspiration, surgical drainage, or catheter drainage (catheters can often be placed by radiologists and may remain in place for days or even weeks) are options. Antimicrobial

therapy should be directed at the anaerobes (especially B. *fragilis*) and the Enterobacteriaceae, and should be started immediately after appropriate specimens are obtained for culture.

B. Clostridium *difficile* colitis: C. *difficile* is the most common cause of diarrhea associated with the use of antimicrobial agents. C. *difficile* is present in 2-5% of the general population, and present in a higher proportion of hospitalized persons. Although infection is endogenous in most cases, hospital outbreaks have established that the environment can be the source as well. Spores can be detected in hospital rooms of infected patients. Alteration of the normal gut flora with antimicrobials favors C. *difficile* by allowing strains that are resistant to the antimicrobial to overgrow, and by favoring the survival of spore-forming bacteria. The disease occurs if the organism proliferates in the colon and produces its toxins, an enterotoxin (toxin A) and cytotoxin (toxin B). Toxin A causes the disruption of intercellular tight junctions followed by altered membrane permeability and fluid secretion. Toxin B causes destruction of the cellular cytoskeleton. The precise role that each toxin plays in the pathogenesis of the disease is still unclear, but the two toxins appear to act synergistically.

C. *difficile* diarrhea usually begins 5 to 10 days into antibiotic treatment. It may be mild and watery or bloody and accompanied by abdominal cramping, leukocytosis, and fever. Severe cases may be associated with an intense inflammatory response. The colonic mucosa becomes studded with inflammatory plaques, which coalesce into an overlying "pseudomembrane" composed of fibrin, leukocytes, and necrotic colonic cells.

Diagnosis of C. *difficile* colitis depends upon the direct detection of toxins in the stool. Treatment involves discontinuing an implicated antimicrobial agent, and/or treatment with oral metronidazole or vancomycin. Relapses or re-infections requiring re-treatment occur in as many as 20% of patients because only the vegetative forms of C. *difficile*, and not its spores, are killed by antibiotics.

Over the last few years, a new strain of C. *difficile* has become prevalent in the United States, Canada, and United Kingdom. C. *difficile* NAP-1 (North America Pulsed-field gel electrophoresis type 1) generates 16-23 times more toxin than other strains, and has been associated with a higher risk of acute clinical decompensation and poor response to metronidazole therapy.

C. Clostridial myonecrosis (Gas gangrene): C. *perfringens* produces a wide range of wound and soft tissue infections, many of which are indistinguishable from other bacterial infections. Clostridial myonecrosis develops in traumatic wounds with muscle damage after contamination with dirt or foreign material containing C. *perfringens* or another species of histotoxic clostridia (C. *histolyticum*, C. *noyyi*, C. *septicum*, and C. *sordellii*). The clostridia can come from spores in the environment or the patient's own intestinal flora. Low oxidation-reduction potential in a wound favors spore germination and multiplication resulting in production of toxins (α-toxin and θ-toxin), which promotes vascular permeability and edema. Intense pain and a sense of heaviness or pressure develops at the site of injury within 1 to 4 days after inoculation, and progresses rapidly

to extensive muscle necrosis and shock, frequently within 2 days of onset. Macroscopic examination of the muscle reveals devitalized necrotic tissue with gas caused by the metabolic activity of rapidly dividing bacteria. Microscopic examination reveals many gram-positive bacilli and the absence of inflammatory cells, as a result of lysis by clostridial toxins. Laboratory diagnosis usually plays only a confirmatory role since treatment must be initiated immediately. Despite all therapeutic efforts, prognosis is poor, and mortality has been reported as high as 40-100%. Myonecrosis must be treated with surgical debridement and high dose penicillin. The utility of hyperbaric oxygen treatment is uncertain, and antiserum against α-toxin have proven to be unsuccessful.

D. Tetanus: C. *tetani* is extremely sensitive to oxygen; however, spore formation allows the organism to survive in the most adverse conditions. It is found in the soil (especially soil treated with manure) and colonizes the gastrointestinal tracts of many animals. The spores are introduced into wounds contaminated with soil or foreign bodies. In developing countries, tetanus often develops in recently delivered infants when the umbilical cord is severed in a nonsterile manner. In an area of low oxidation-reduction potential, such as a necrotic wound, C. *tetani* multiply locally and elaborate Tetanospasmin. The neurotoxin enters the presynaptic terminal of lower motor neurons and reaches the central nervous system by exploiting the retrograde axonal transport system. In the anterior horn cells of the spinal cord, Tetanospasmin blocks the postsynaptic inhibition of spinal motor reflexes resulting in spasmodic contractions of both protagonist and antagonist muscles. Duration between time of inoculation and onset of neurological symptoms ranges from a few days to weeks depending upon the distance of primary wound infection to the CNS.

The most common clinical manifestation is generalized tetanus, which involves sustained contraction of the masseter muscles (trismus or lock-jaw) or facial muscles (sardonic smile). Other early signs are drooling, sweating, irritability, and persistent back spasms. The autonomic nervous system is involved in patients with more severe disease. Localized tetanus involves only the musculature at the site of the primary infection. Neonatal tetanus is typically associated with an initial infection of the umbilical stump that becomes generalized. Diagnosis is made upon the basis of clinical presentation. Microscopic detection of C. *tetani* is rare, and cultures are usually negative since the organisms are extremely sensitive to oxygen. Neither the tetanus toxin nor the antibodies are detectable in the patient.

Treatment of tetanus requires debridement of the primary wound and treatment with metronidazole to eliminate remaining vegetative bacteria, passive immunization with human tetanus immunoglobulin to bind free tetanospasmin, and vaccination with tetanus toxoid. Since toxin bound to nerve endings is protected from antibodies, the patient must be supported until normal regulation of synaptic transmission is restored. In the United States, because of the high prevalence of immunity from toxoid vaccination, fewer than 50 cases are reported annually (primarily in elderly with waning immunity).

E. Botulism: C. *botulinum* is commonly isolated in soil and water. There are 7 distinct botulinum toxins and human disease is associated with types A, B, E, and F. Individual

isolates typically produce only one toxin. Like the tetanus toxin, Botulinum toxin consists of a neurotoxin subunit (A or light chain) and one or more non-toxic subunits (B or heavy chain), to protect the neurotoxin from being inactivated by stomach acids. Botulinum toxin is very specific for cholinergic nerves, and blocks neurotransmission at peripheral cholinergic synapses by preventing release of acetylcholine. The two most common presentations of botulism are infant botulism and foodborne botulism.

Infant botulism is the most common form of botulism in the United States (100 cases/year) and is caused by the neurotoxin produced by C. *botulinum* colonizing the infant's gastrointestinal tract. C. *botulinum* can survive in the infant's gastrointestinal tract, but not in the adult gastrointestinal tract. The disease typically affects infants less than 1 year old, especially those exposed to honey. Symptoms are usually nonspecific, but progressive disease may develop (flaccid paralysis, respiratory arrest).

Food-bourne botulism usually starts 12-36 hours after ingestion of the toxin. Botulism most often occurs after ingestion of home-canned products that have not been heated at temperatures sufficient to kill C. *botulinum* spores. The alkaline conditions provided by canned vegetables and fish support the conversion from spore to vegetative state, to multiply and produce toxin. This may occur with no change in food taste, color or odor. Because the toxin is heat labile, food must be ingested un-cooked or inadequately-cooked. The initial signs are blurred vision, dry mouth, constipation, and abdominal pain. Fever is absent. Bilateral descending weakness of the peripheral muscles develops in patients with progressive disease (flaccid paralysis), with paralysis of the respiratory muscles as the most serious consequence. Since the neurotoxin binds irreversibly, complete recovery is dependent upon regeneration of affected nerve endings, and may require months to years.

Clinical diagnosis of botulism is confirmed if the organism if isolated (culture of implicated food or feces) or toxin activity is demonstrated (inoculation of blood, intestinal contents or food into mice). Treatment involves intensive supportive measures (mechanical ventilation), elimination of gastrointestinal carriage of organism (gastric lavage, metronidazole), and botulinum antitoxin to bind circulating toxin.

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