BACTERIOLOGY - CHAPTER FIFTEEN

ANAEROBES AND *PSEUDOMONAS* - OPPORTUNISTIC INFECTIONS

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ANAEROBES

Obligate anaerobes are bacteria that cannot survive in the presence of a high oxidation-reduction potential (redox potential) / high oxygen content. During metabolism, bacteria can produce toxic bi-products from oxygen (including superoxide radicals and hydrogen peroxide). Strict anaerobes lack certain enzymes (including superoxide dismutase and catalase) that detoxify these products.

Polymicrobic anaerobic infection

Strict anaerobes cannot grow in healthy tissues due to their oxygen content. When tissue injury occurs with limitation of the blood (and oxygen) supply, conditions are created for opportunistic growth of obligate anaerobes. Often more than one species will infect the same site. Simultaneous infection with a facultative anaerobe (which uses up the already diminished oxygen supply) also encourages growth of obligate anaerobes.

Endogenous versus exogenous infection

Most anaerobes in the normal flora are non-spore formers and anaerobic infections often occur from this source. However, contamination of wounds can also occur with anaerobic spore-formers (e.g. clostridia) which are common in the environment (e.g. soil). Non-spore-formers rarely produce exotoxins in contrast to spore-formers.

Sites of anaerobes in normal flora

Strict anaerobes are present in large numbers in the intestine (95 to 99% of total bacterial mass), but also in the mouth and genitourinary tract. The most common infections resulting from abdominal surgery or other gut injury are *Enterobacteriaceae* (facultative anaerobes) and *Bacteroides fragilis* (see below). These are minor components of the gut flora and demonstrate the important point that certain organisms more readily produce opportunistic infections than others.

Problems in identification of anaerobic infections

- They are often derived from the normal flora. One must be confident that one has not isolated a contaminant.
- If air gets into the sample during sampling or transportation to the clinical laboratory, then the organism may not be isolatable.
- Slow growth of the organism (due to inefficiency of fermentation) means isolation takes several days or longer.

KEY WORDS
Anaerobes
Oxidation-reduction (redox) potential
Catalase
Superoxide dismutase
Polymicrobic (mixed) infection
Spore formers
Non-spore formers
Bacteroides
B. fragilis
Clostridium tetani
Tetanospasmin
C. perfringens
Lecithinase (phospholipase, alpha toxin)
C. perfringens enterotoxin
C. botulinum
Botulinum toxin
C. difficile
C. difficile enterotoxin
Pseudomonas aeruginosa
Pyocyanin
Fluorescein
Toxin A


Figure 1a Gram-stained Gram-positive *Clostridium tetani* bacteria, which had been cultivated on a blood agar plate. *C. tetani* is a slender,

Identification in the clinical laboratory after isolation

Two systems are commonly used: Biochemical systems and/or gas chromatographic identification of volatile fermentation products (short chain fatty acids / alcohols)

ANAEROBIC NON-SPORE-FORMERS OF CLINICAL IMPORTANCE

- Gram-negative rods (*Bacteroides* [e.g. *B. fragilis*] and *Fusobacterium)*
- Gram-positive rods (*Actinomyces*, *Arachnia*, *Eubacterium*, *Bifidobacterium*, *Lactobacillus*, *Propionibacterium)*
- Gram-positive cocci (*Peptostreptococcus* and *Peptococcus)*
- Gram-negative cocci (*Veillonella*, *Acidominococcus*)

Bacteroides fragilis

B. fragilis is the most important strict anaerobic non-spore-former causing clinical disease. It has a prominent capsule that is involved in pathogenesis since it is:

- Anti-phagocytic
- Directly involved in abscess formation

This bacterium also produces an endotoxin which differs in composition from typical endotoxin and is of low toxicity.

- Case report: Multidrug-Resistant *Bacteroides fragilis* - Seattle, Washington, 2013

ANAEROBIC SPORE-FORMERS (CLOSTRIDIA)

These are Gram-positive rods. They are found in the environment (particularly soil) but also intestine of man and animals.

Tetanus

Clostridium tetani

Clostridium tetani, a gram-positive rod that forms a terminal spore (figure 1a and b), is commonly found in the soil, dust and animal feces. Contamination of wounds, which provide anaerobic conditions, can lead to spore germination and tetanus, a relatively rare (in western countries) but frequently fatal disease. Death occurs in about 11% of cases with most of these in the more elderly patients (over 60 years of age). Tetanus is also know as lockjaw because of the patient's inability to open the mouth as a result of muscle paralysis. The rarity of the disease results from an excellent vaccine and most cases that are now seen in the United States are in adults who never received the vaccine. Thus, currently some 60% of cases are in adults over 50 (figure 2). In the period 1947 to 2008, tetanus cases in the US dropped by over 95% and deaths by over 99% (figure 3). From 2000 through 2009 an average of 29 cases were reported per year in the United States. Vaccination has reduced neonatal tetanus in developed countries so that in the United States there have been just two cases since 1989. Both patients were born to unvaccinated mothers.

In third world countries, many (about half) of tetanus cases are in neonates where the unhealed umbilical stump becomes infected, often as a result of cutting the umbilical cord with a contaminated knife. Many neonatal deaths result (about 270,000 in 1998). This occurs when the mother has no protective immunity to pass on to the infant

Infection usually occurs when spores (in dirt, feces or saliva) enter wounds and scratches where they germinate and produce tetanus toxin. Puncture wounds,

anaerobic rod that may develop a terminal spore, giving it a drumstick appearance. CDC/Dr Holdeman


Figure 1b *C. tetani*. Note terminal spores. CDC

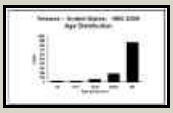
Figure 2a Tetanus cases by age 1980-2000 CDC

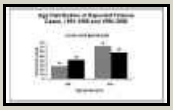
Figure 2b Age distribution of reported tetanus cases 1991-1995 and 1996-2000 CDC

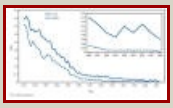
Figure 3 Annual rate of tetanus cases and tetanus deaths in the United States during 1947–2008. From 1947–2008, the number of tetanus cases reported each year, which already had decreased greatly since 1900, continued to decline. CDC

such as by a needle or nail, other wounds and scratches and burns can all lead to *C. tetani* tinfections. More rarely, surgical procedures and dental extractions can lead to tetanus. Tetanus can also be contracted from the use of intravenous drugs.

The organism is non-invasive and thus remains in the local wound. The exotoxin (tetanospasmin) binds to ganglioside receptors on inhibitory neurones in central nervous system in which glycine is commonly the neurotransmitter. This stops nerve impulse transmission to muscle leading to spastic paralysis. The toxin can act at peripheral motor nerve end plates, the brain, spinal cord and also in the sympathetic nervous system. Because inhibitory neurons are involved, the result is unopposed muscle contraction.

In generalized tetanus, the most common form, the patient typically experiences lockjaw (trismus). This is a stiffness of the jaw muscles that results in inability to open the mouth (figure 4) or swallow leading to the appearance of a sardonic smile (*risus sardonicus*) (figure 5). Speech as a result of spasm of the vocal cords may be affected. Continued severe muscle contractions (figure 6 and 7), which can even cause broken bones, and resulting spasms, often lasting for minutes over a period of weeks, can be fatal. The patient often experiences headaches and/or a fever (a rise of 2 to 4 degrees) with sweating, elevated heart rate and blood pressure. Tetanus patients in hospital often experience nosocomial infections. Aspiration pneumonia is often a late complication.

On average, about eight days after infection symptoms of tetanus appear (though the incubation period can be a short as three days and as long as thee weeks). The incubation period seems to depend on the distance of the infection site from the central nervous system. In neonates the average latent period is about a week.

Other forms of tetanus

Cephalic tetanus is a rare infection involving the middle ear. It can affect cranial nerves.

Local tetanus is also rare and manifests itself as localized muscle contractions in the area of infection. Few cases of local tetanus are fatal.

Vaccination

Vaccination of infants with tetanus toxoid have almost eliminated this disease in the United States. The toxoid consists of tetanus toxin that has been inactivated using formalin that stimulates anti-toxin antibodies. It comes in two forms: precipitated and fluid. The precipitated form yields more rapid seroconversion and higher anti-toxin titers. If infants receive the complete vaccination regimen, virtually 100% protection is achieved. Boosters should be given every ten years.

Diagnosis

Diagnosis is clinical and bacteria are only derived from wounds in a minority of cases.

Treatment

Tetanus is an emergency situation and requires hospitalization. The patient is immediately treated with human tetanus immune globulin (or equine antitoxin). Drugs can control muscle spasms. The wound requires aggressive washing and treatment with antibiotics.


Figure 4. This baby has tetanus. He cannot breast feed or open his mouth because the muscles in his face have become so tight WHO

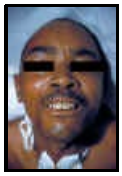
Figure 5. Patient with facial tetany. Note the contraction of the masseter and neck muscles CDC/Dr. Thomas F. Sellers/Emory University


Figure 6. This baby has neonatal tetanus. It is completely rigid. Tetanus kills most of the babies who get it. Infection usually happens when newly cut umbilical cord is exposed to dirt CDC


Figure 7. Severe case of adult tetanus. The muscles in the back and legs are very tight. Muscle spasms can break bones CDC


Figure 8. Neonatal tetanus summary CDC


Figure 9. Tetanus - clinical features CDC


Figure 10. Tetanus: Injuries and conditions CDC

Gas Gangrene

Clostridium perfringens

Clostridium perfringens, a gram positive rod (figure 11), causes wound colonization (gas gangrene) after soil, and to a lesser extent intestinal tract, contamination. It is primarily seen in time of war as a result on non-sterile field hospitals and projectile wounds. The term gas gangrene refers to swelling of tissues due to release of gas, as fermentation products, of clostridia. Progression to toxemia and shock is frequently very rapid.


Figure 11a. Gas Gangrene. Gram stain.


Figure 11b. Clostridium sp. Gram-positive stain. CDC.


Figure 12. Gas gangrene of leg

Gas gangrene can easily be identified by the large, blackened sores (figure 12) and loud and distinctive sound (crepitus) caused by gas escaping from necrotic tissue. It is a moist gangrene, in contrast to dry gangrene which is not caused by a bacterial infection.

The organism produces several tissue degrading enzymes (including lecithinase [alpha toxin], proteolytic and saccharolytic enzymes). Necrosis and destruction of blood vessels and the surrounding tissue, especially muscle, result (myonecrosis is a condition of necrotic damage, specific to muscle tissue). This creates an anaerobic environment in adjacent tissue and the organism spreads systemically. Death can occur within two days. Nowadays, treatment (including anti-toxin, antibiotic therapy, debridement) is extremely effective and amputation and death is rare.

The determination of production of lecithinase is important in laboratory identification of the organism.

This bacterium is also a significant cause of food poisoning by enterotoxin producing strains.

Botulism

Clostridium botulinum

Botulism (a rare but fatal form of food poisoning) is caused by a potent nerve exotoxin (botulinum toxin). It is a serious paralytic illness caused by *Clostridium botulinum* (figure 13) and, more rarely, by strains of *Clostridium butyricum* and *Clostridium baratii*.

WEB RESOURCES

CDC tetanus manual (requires Acrobat)

CDC information on botulism

CDC botulism manual (requires Acrobat)

Seminal facts about botulism (from WHO)

Bacterial toxins: Friend or

Foe (from CDC)


Figure 13a Gentian violet stain of *C. botulinum*.


Figure 13b *Clostridium botulinum* - rod prokaryote. Vegetative (yellow arrow) and spore (blue arrow) stages : note the flagella on the vegetative cells. Causes botulism. SEM x15,400,

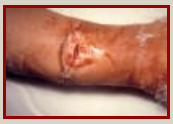
Figure 14 Wound botulism involvement of compound fracture of right arm. 14-year-old boy fractured his right ulna and radius and subsequently developed wound botulism. CDC


Figure 15a Morphology of Gram-positive *Clostridium difficile* bacillus. CDC


Figure 15b *C. difficile* from a stool sample culture. CDC

The toxin (of which there are seven types, designated as A through G but only types A, B, E and F cause illness in humans) binds to receptors on peripheral nerves, where acetylcholine is the neurotransmitter and inhibits nerve impulses. Flaccid paralysis and often death (from respiratory and/or cardiac failure) ensue. The organism does not grow in the gut, but pre-formed exotoxin from prior germination of spores may be present in inadequately autoclaved canned food (usually at home).

Besides food poising, *C. botulinum* can cause:

- Wound botulism (figure 14) but is even rarer than botulism food poisoning.
- In addition, iatrogenic botulism can occur from accidental overdose of botulinum toxin.

C. botulinum does not readily grow in the adult intestine due to competition with the normal flora and their requirement for an anaerobic, low acidity environment. In infants, where the flora is not established, colonization with *C. botulinum* can occur. Infant botulism, although uncommon, is now the predominant form of botulism. In the United States, there are about 150 cases of botulism per year of which three quarters are infant botulism and 15% come from contaminated food (usually home-reserved food). The remainder are wound botulism (mostly associated with black-tar heroin injection). The spores can remain viable for many years.

Symptoms

After eating contaminated food, the symptoms of botulism occur usually with a day or two but sometimes there may be a period of up to a week before they appear. Vision and swallowing are affected and the patient may become nauseated and constipated. Muscle paralysis ensues, usually starting at the head and, when the respiratory muscles are affected, death can result. While the bacterium does not grow in the adult large intestine, it can in infants who ingest spores that are ubiquitous in the environment - Eating honey contaminated by spores is one source. Again, an early symptom is constipation and general malaise. With muscle paralysis, swallowing becomes difficult and paralysis of the head muscles leads to the characteristic floppy baby symptoms. Impaired respiratory muscles lead to breathing difficulties and possibly to death. When severe wounds are infected, the conditions are right for the growth of clostridia leading to similar symptoms to food-borne botulism except that the gastro-intestinal tract is not involved.

Treatment

Treatment for adults includes an enema to clear the gastro-intestinal tract of the toxin and injection of anti-toxin (antibodies produced in horses). It is important that the anti-toxin is given early to neutralize the toxin and protect nerve endings from damage. The horse-derived anti-toxin is not used in infants who receive, instead, human botulism immune globulin. Antibiotics are not used to treat botulism, although they may be used in secondary infections, because of the possibility of more toxin being released as bacteria are lyzed. Supportive treatment of infants is based on helping them breath and on tube feeding. Adults may also require a respirator and possibly a tracheotomy and intensive medical and nursing care for several months.

Death from botulism has become much rarer in the past 50 years. The proportion of patients with botulism who die has fallen from about 50% to 3 to 5%. Some patients die from infections or other problems as a result of being paralyzed for weeks or months. Patients who survive an episode of botulism poisoning may have fatigue and shortness of breath for years and long-term therapy may be needed to aid recovery. However, complete recovery from botulism usually occurs over a period of months as the damaged nerve endings are replaced.

Clostridium difficile

C. difficile is frequently a nosocomial infection. The organism, a gram positive rod

(figure 15), can cause a variety of diseases including:

- pseudomembranous colitis, a form of gastroenteritis
- toxic megacolon
- perforations of the colon
- sepsis

Patients at elevated risk include those that have received:

- antibiotics
- proton pump inhibitors
- gastrointestinal surgery/manipulation
- long length of stay in healthcare settings
- a serious underlying illness

Those with immunocompromizing conditions and advanced age are also at high risk

However, *C. difficile* infection is rarely fatal.

Symptoms

When the normal flora of the intestine is altered by antibiotic therapy, this organism - which is present in the gastro-intestinal tract of many babies - can grow and colonize. *C. difficile* produces an enterotoxin and pseudomembranous colitis can result. Symptoms, which include abdominal cramps and watery diarrhea, start some days (4 to 8) after initiation of antibiotic therapy. In mild cases, there is no blood in the diarrhea but, in severe cases, bloody diarrhea, a distended tender abdomen and fever can occur.

Treatment

Therapy includes discontinuation of the implicated antibiotic (e.g. ampicillin). Severe cases require specific antibiotic therapy (e.g. with vancomycin).

Figure 16
Scanning Electron Micrograph of *Pseudomonas aeruginosa*
CDC

Figure 17
Three-dimensional computer-generated image of four multidrug-resistant *Pseudomonas aeruginosa* bacteria. The artistic recreation was based upon scanning electron micrographic imagery. Note the presence of numbers of thin, diaphanous fimbriae emanating from the organisms' cell wall, as well as a single, corkscrew-shaped flagellum, which provides for the bacteria's unipolar mode of motility.
CDC/Melissa Brower

Figure 18
Colorized scanning electron micrograph of a number of *Pseudomonas aeruginosa* bacteria.
CDC/ Janice Haney Carr

PSEUDOMONAS AERUGINOSA

Pseudomonads are aerobic, gram-negative rods with polar flagella. They are oxidase positive, in contrast to *Enterobacteriaceae*. These organisms are found in most environments including in water and soil and air. Among the genus *Pseudomonas,* the majority of human infections are caused by *P. aeruginosa* (figure 16 and 17), although other related organisms also cause disease. Normally, individuals with compromised immune systems such as those infected with HIV, organ transplant recipients and burns patients are particularly prone to pseudomonad infections and mortality can be high (e.g. as much as 90% in heart infections). In burns and wounds, there is destruction of blood vessels which limits access of phagocytes that would normally clear the region of the pathogen. Cystic fibrosis patients are also at risk for infection since alteration of the respiratory epithelium commonly allows colonization and development of pneumonia. This is often seen in children who may suffer recurrent bouts of pseudomonad pneumonia resulting in fever, a wheezing productive cough, distended abdomen, breathing difficulties and cyanosis. This is often accompanied by weight loss.

Pseudomonads are opportunistic pathogens. Nosocomial infections by *P. aeruginosa* are particularly common in intensive care units and can lead to fatal pneumonia in which the patient has a productive cough, chills, breathing difficulties and cyanosis. The problem is compounded by the often encountered resistance of pseudomonads to common antibiotics. Moreover, the slime layer that is produced over the surface of these organisms has an anti-phagocytic effect making their control by the immune system phagocytes difficult; yet, they stick readily to other cells. They produce tissue-damaging toxins.

Infections by *P. aeruginosa* are a common cause of bacteremia, that is bacterial blood infections. Heart valves, particularly of intravenous drug users, can also become infected. Symptoms include general malaise with fever with joint and muscle pain.

Pseudomads can infect the skin as a result of bathing in infected waters, resulting in a

itching rash in otherwise healthy individuals. This is the so-called "hot tub folliculitis". Sometimes these skin infections can be severe and result in headache, sore eyes, stomach and breast pain and earache. Injury can lead to infections of soft tissues and of bone and joints and the bacteria can also spread to these sites from a bacteremia. Bone involvement is sometimes seen in diabetics as well as persons who are undergoing surgery. Infection of wounds can result in the characteristic fruity smell and blue-green secretions (pyocyanin).

Among other pseudomonad-caused infections are those of the urinary tract, often as a result of catheter use or surgery, the brain which can develop abscesses and meningitis, and the eyes and ears. Swimmer's itch is an innocuous infection of the ear canal by these bacteria but older patients can experience life-threatening infections of the ear which sometimes cause paralysis of facial muscles. Abrasion of the cornea can lead to infection and resultant corneal ulcers which, if left untreated, can cause severe damage and loss of sight. Some eye medications and prolonged use of soft contact lenses can exacerbate the infection.

Identification of a pseudomonad infection includes pigment production: pyocyanin (blue-green) and fluorescein (green-yellow, fluorescent) and biochemical reactions (oxidase test). Cultures have fruity smell. Since hospitals are so commonly infected with pseudomonads, the presence of the organism is not sufficient to prove it as a source of the infection. Techniques such as X-rays can be used to assess deep tissue and bone infections.

Resistance of pseudomonads to various antibiotics is a problem. Two such drugs simultaneously are often employed for up to 6 weeks, either by mouth or intravenously. Eye infections are treated with antibiotic drops. In the case of infections of deep tissues such as in the brain, joints or bone, surgery to remove damaged tissue may be required. Moreover, amputation may be necessary in infections of the limbs of burns patients or those with infected wounds.

The toxicity of pseudomonads results from production of Toxin A which ADP ribosylates elongation factor-2 (EF2 - used in protein synthesis). In this, pseudomonad toxin is similar to diphtheria toxin

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