

Lecture 47: Anaerobes and *Pseudomonas*

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Suggested reading: Murray, 6th Edition Chapter 40 and 41 (anaerobe non-spore formers), Chapter 39 (clostridia, spore former), Chapters 33 (*Pseudomonas*, an aerobe)

BRIEF OUTLINE OF MAJOR TEACHING POINTS

1. Overview of anaerobic bacteriology
2. Anaerobic non-spore formers
3. Anaerobic spore formers (clostridia)
4. *Pseudomonas* (a strict aerobe)

KEY WORDS

Obligate anaerobes	Lecithinase (phospholipase, $\alpha$ toxin)
Strict anaerobes	<i>C. perfringens</i> enterotoxin
Polymicrobial (mixed) infection	<i>C. botulinum</i>
Spore formers	Botulinum toxin
Non-spore formers	<i>C. difficile</i>
<i>Bacteroides</i>	<i>C. difficile</i> enterotoxin
<i>B. fragilis</i>	<i>Pseudomonas aeruginosa</i>
<i>Clostridium tetani</i>	Pyocyanin
Tetanospasmin	Fluorescein
<i>C. perfringens</i>	Toxin A

**1. ANAEROBES (Obligate anaerobes, strict anaerobes)**

Obligate anaerobes are bacteria that can't survive in the presence of high oxidation 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.

Polymicrobial anaerobic infection

Strict anaerobes can't grow in healthy tissues due to its 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) that 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:

Anaerobes are commonly found on most body surfaces. Thus opportunistic infections throughout the body are common with muscle and cutaneous/sub-cutaneous necrosis and abscesses being particularly observed. Strict anaerobes are present in large numbers in the intestine (95-99% 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 then *B. fragilis* (see below) although both are minor components of the gut flora.

### Problems in identification of anaerobic infections

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

### Identification in the clinical laboratory after isolation

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

## **A. ANAEROBIC NON-SPORE-FORMERS OF CLINICAL IMPORTANCE**

1. Gram-negative rods (*Bacteroides* [e.g. *B. fragilis*] *Fusobacterium*), *Porphyromonas*, *Prevotella*.

2. Gram-positive rods (*Actinomyces*, *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, *Mobiluncus*, *Propionibacterium*).
3. Gram-positive cocci (*Peptostreptococcus* and *Peptococcus*).
4. Gram-negative cocci (*Veillonella*).

### *Bacteroides fragilis*

1. The most important strict anaerobic, non-spore-former that causes clinical disease.
2. Prominent capsule involved in pathogenesis: a) anti-phagocytic and b) directly involved in abscess formation.
3. Endotoxin that differs in composition from typical endotoxin and is of low toxicity.
4. Produces a beta lactamase (as do many other strict anaerobes of clinical significance).

## **B. ANAEROBIC SPORE-FORMERS (CLOSTRIDIA)**

Gram-positive rods, reservoir: found in the environment (particularly soil) but also intestine of man.

### *C. tetani*

1. Commonly found in the soil; thus contamination of wounds can lead to colonization and tetanus.
2. The exotoxin (tetanospasmin) binds to ganglioside receptors on inhibitory neurones in CNS (where glycine is commonly the neurotransmitter) and stops nerve impulse transmission to muscle leading to rigid (spastic) paralysis. The toxin causes continued severe muscle contractions and spasms result that can be fatal. Tetanus toxin is an A-B toxin where the A chain is a peptidase.

3. The organism is non-invasive and thus remains in the local wound.

4. Vaccination of infants with tetanus toxoid (DPT, diphtheria, pertussis and tetanus) has almost eliminated this disease in the US.

### *C. perfringens*

1. Causes wound colonization (gas gangrene) after soil, and to a lesser extent intestinal

tract, contamination. Gangrene is primarily seen in time of war. The term gas gangrene refers to swelling of tissues due to release of gas, as fermentation products, of clostridia.

2. The organism produces several tissue degrading enzymes (including lecithinase [ $\alpha$  toxin], proteolytic and saccharolytic enzymes); necrosis and destruction of blood vessels and surrounding tissue result. This creates an anaerobic environment in adjacent tissue and the organism spreads systemically. Death can occur within 2 days. Nowadays, treatment (including anti-toxin, antibiotic therapy, debridement) is extremely effective and amputation and death is rare.

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

4. A significant cause of food poisoning by enterotoxin producing strains.

#### *C. botulinum:*

1. Botulism (a rare but fatal form of food poisoning) is caused by a potent exotoxin (botulinum toxin). This toxin binds to receptors on peripheral nerves, where acetylcholine is the neurotransmitter. The toxin inhibits nerve impulses and flaccid paralysis results and often death (from respiratory and/or cardiac failure). The exotoxin is an A-B toxin and the A chain has peptidase activity. 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).

2. Wound botulism can occur but is also rare.

3. *C. botulinum* does not readily grow in the adult intestine due to competition with the normal flora. In the neonate, where the flora is not established, colonization with *C. botulinum* can occur. Infant botulism, although uncommon, is now the predominant form of botulism.

4. Treatment includes administration of anti-toxin and (for neonates) antibiotic therapy.

5. Botulinum toxin is used as a biological warfare agent; not the live agent. Thus this is more like a chemical attack.

#### *C. difficile*

When the normal flora of the intestine is altered by antibiotic therapy, this organism can multiply and pseudo-membranous colitis results. The organism produces exotoxin A (enterotoxin) and exotoxin B (cytotoxin). Therapy includes discontinuation of the implicated antibiotic (e.g. ampicillin). Severe cases require specific antibiotic therapy (e.g. vancomycin).

## 2. PSEUDOMONAS AERUGINOSA

1. Aerobic, gram-negative rods, polar flagella, oxidase positive (in contrast to *Enterobacteriaceae*).
2. Among the genus *Pseudomonas* the majority of human infections are caused by *P. aeruginosa*, although other related organisms also cause disease.
3. *Pseudomonas aeruginosa* is a water-loving organism commonly transmitted through the air. The compromised host is infected e.g. a) Burns and wounds - destruction of blood vessels limits access of phagocytes b) After use of cytotoxic drugs in cancer therapy (which destroys the immune system) c) Alteration of the respiratory epithelium in cystic fibrosis commonly allows colonization and development of pneumonia.
4. Identification includes (a) pigments: pyocyanin (blue-green) and pyoverdinin (green-yellow, fluorescent) and (b) biochemical reactions. Cultures have fruity smell.
5. Slime layer is anti-phagocytic.
6. Toxin A - ADP ribosylates EF2 similar action to diphtheria toxin

Other related opportunists: *Burkholderia*, *Stenotrophomonas*, *Acinetobacter* and *Moraxella*.