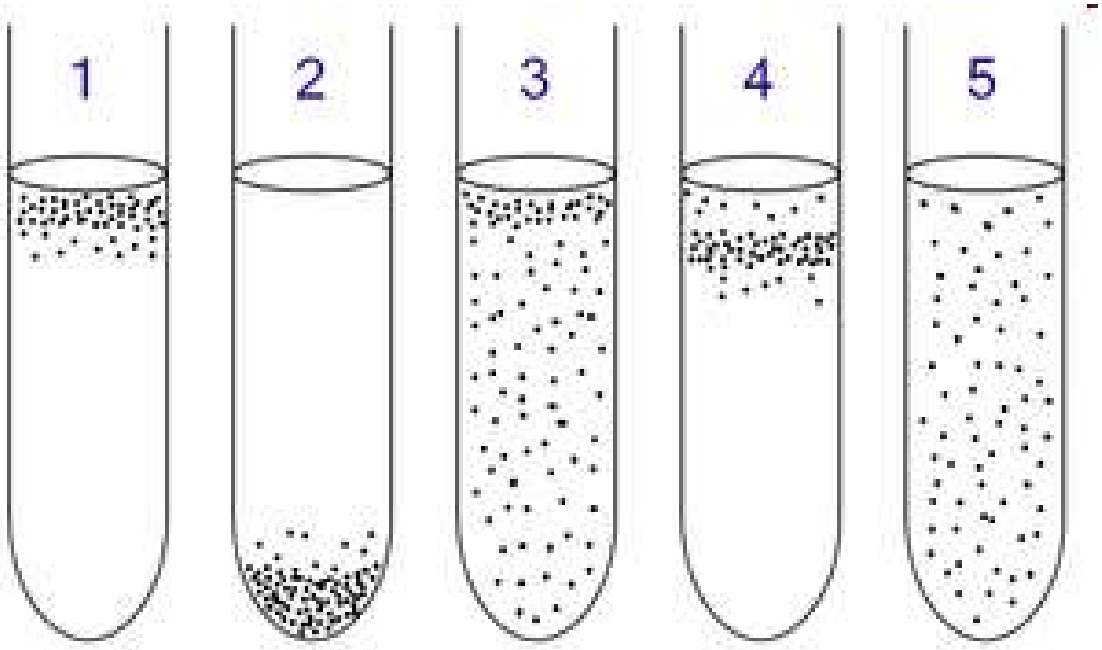
Anaerobic bacteria

Traditional classification of bacteria concerning to oxygen requirement



1. **Obligate aerobic** (oxygen-needing) bacteria grow at the top of the test tube in order to absorb maximal amount of oxygen.

2. **Obligate anaerobic** bacteria grow at the bottom to avoid oxygen.

3. **Facultative anaerobic bacteria** grow mostly at the top, since aerobic respiration is the most beneficial one; but as lack of oxygen does not hurt them, they can be found all along the test tube.

4. **Microaerophiles grow** at the upper part of the test tube but not at the top. They require oxygen but at a low concentration.

5. **Aerotolerant bacteria** are not affected at all by oxygen, and they are evenly spread along the test tube.

Anaerobiosis (anaerobic respiration)

- refers to the oxidation of molecules in the absence of oxygen to produce energy, in opposition to aerobic respiration which does use oxygen. Cell respiration is the process of generating ATP through a process of oxidation and where an inorganic molecule is the final electron carrier.
- Anaerobic respiration processes require another electron acceptor to replace oxygen. Anaerobic respiration is often used interchangeably with fermentation, especially when the glycolytic pathway is used for energy production in a obligate anaerobe cell. They are not synonymous terms. Cellular respiration happens when and organic molecule is the final electron carrier (such as lactic acid, ethanol etc). Anaerobic prokaryotes can generate all of their ATP using an electron transport system and ATP synthase. It is almost as efficient as aerobic respiration but much more efficient than fermentation.

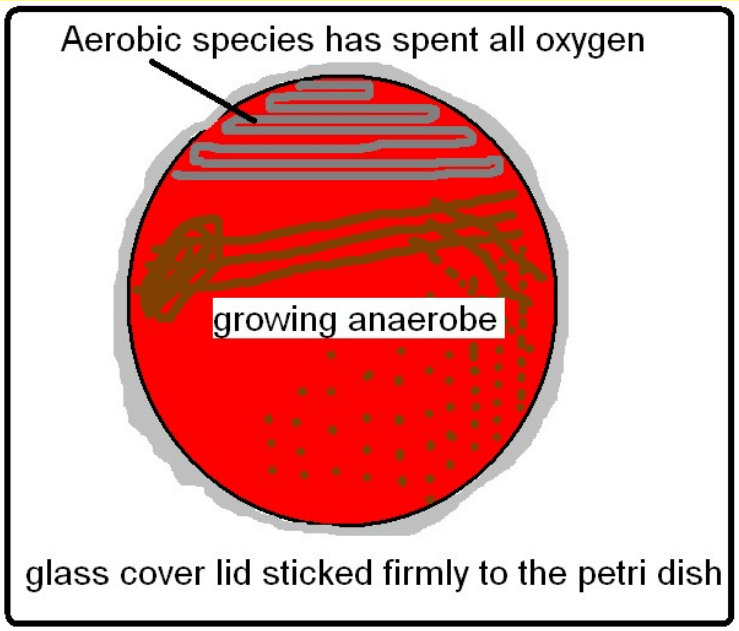
Methods for cultivation of anaerobic bacteria

Oxygen can be „withdraw“ by three main ways

1. Biologically – aerobic species (e.g. *Serratia marcescens*) is inoculated on the same plate as clinical material suspected for appearance of anaerobic bacterial agent, during the cultivation the growing aerobe spent all oxygen and the anaerobe can grow).

2. Physically – using a vacuum system oxygen is evacuated before cultivation start and replaced by artificial gas

3. Chemically – using a chemical reaction oxygen is spent and anaerobes can grow



Biological anaerobiosis (an obsolete and time consuming method not more used today)

Today´s method for cultivation of anaerobes

Anaerostat

• Oxygen is evacuated before cultivation start and replaced by artificial gas
• Temperature 37C
• Culture media (e.g. Schaedler agar) are inoculated by clinical material and cultured (48 hours)

Clostridium tetani

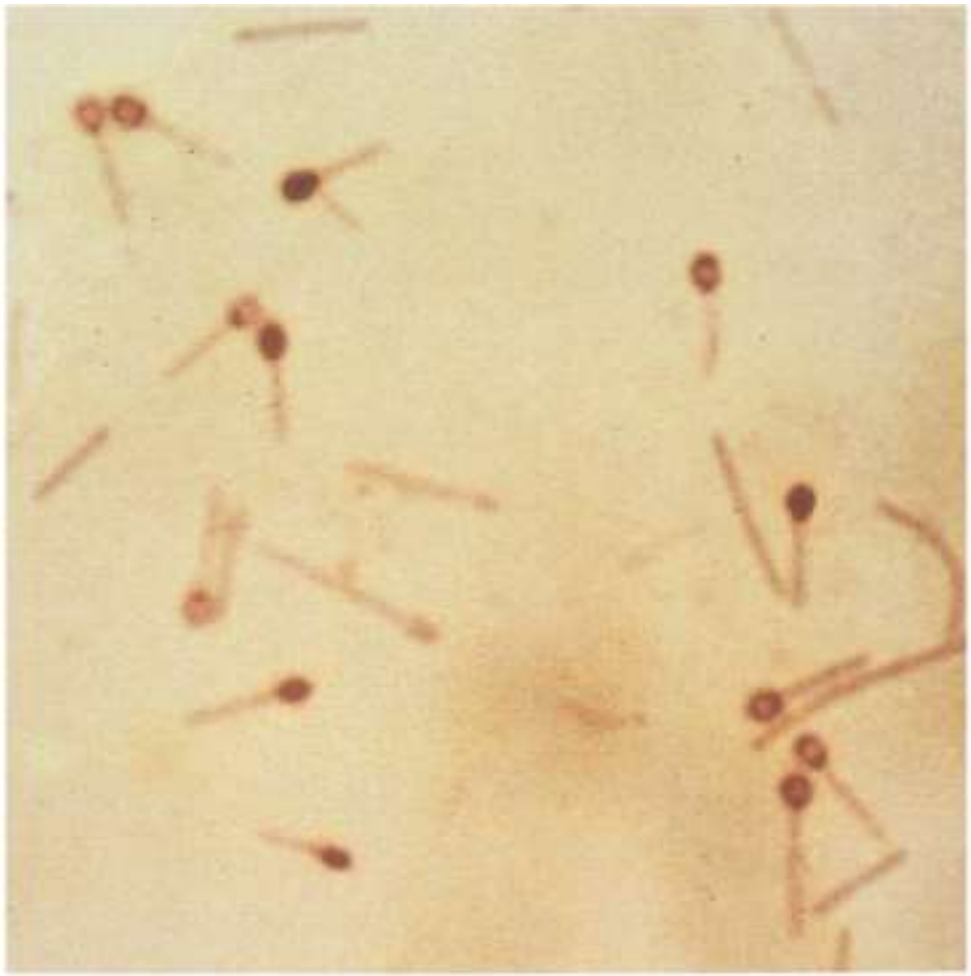
Physiology and Structure
* **Gram-positive rods** with prominent terminal **spores** (drumstick appearance)
* **Strict anaerobe** (vegetative cells are extremely oxygen sensitive)
* **Difficult to isolate from clinical specimens (don´t be late with diagnosis)**

Virulence
* Spore formation
* **Tetanospasmin** - heat-labile **neurotoxin**; **blocks release of neurotransmitters**
* **Tetanolysin** (heat-stable hemolysin of unknown significance)

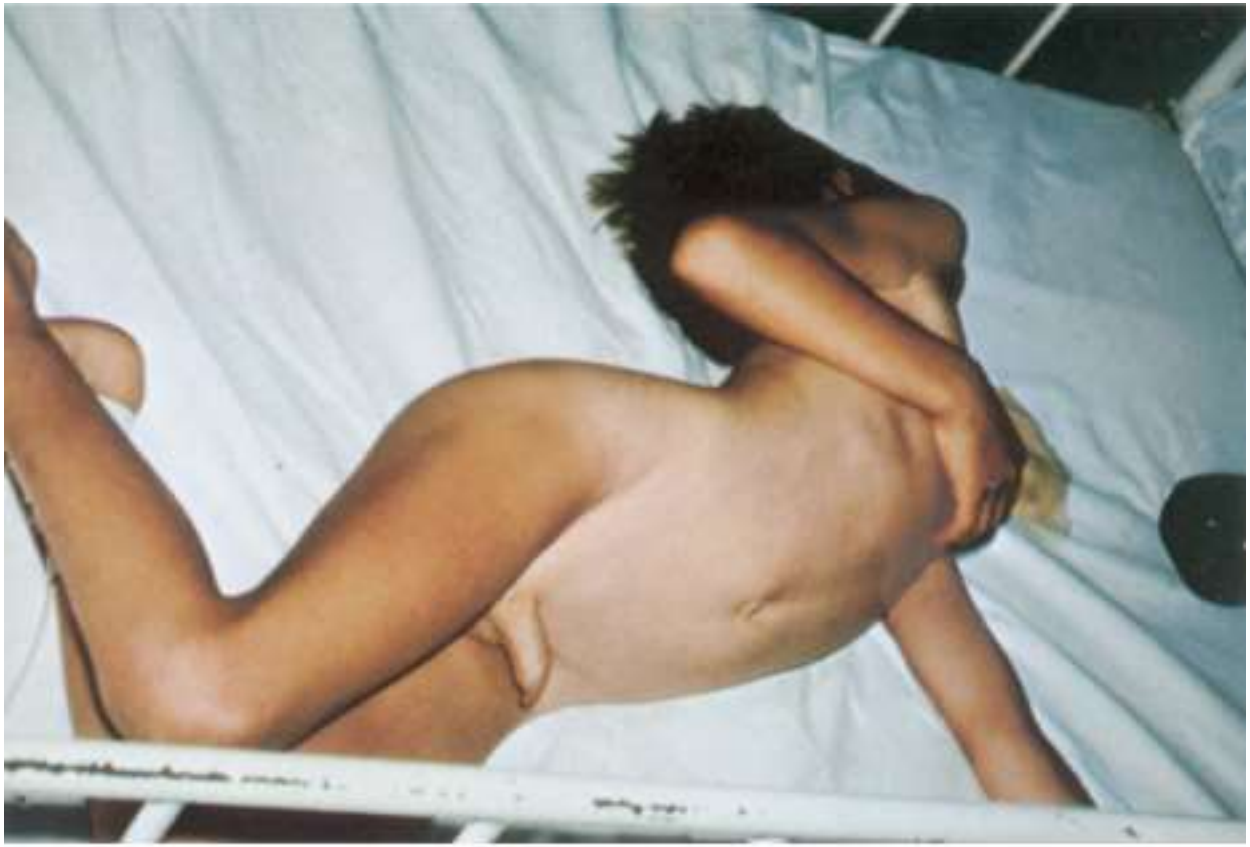
Epidemiology
* **Ubiquitous; spores are found in most soils and can colonize gastrointestinal tract of humans and animals**
* Exposure to spores is common, but disease is uncommon, except in developing countries where there is poor access to vaccine and medical care
* **Risk is greatest for people with inadequate vaccine-induced immunity**; disease does not induce immunity
* Diagnosis is **based on clinical presentation**
* **Microscopy and culture with poor sensitivity**
* Neither tetanus toxin by PCR nor antibodies are typically detected

Treatment, Prevention, and Control. Treatment **requires débridement, antibiotic therapy (penicilin G + clindamycine), passive immunization with antitoxin globulin, and vaccination with tetanus toxoid**
* Prevention - vaccination, consisting of **three doses of tetanus toxoid**, followed by booster doses every 10 years

Gram staining of *Clostridium tetani and terminally located (drumstick) spores* (Murray et al., Medical Microbiology)



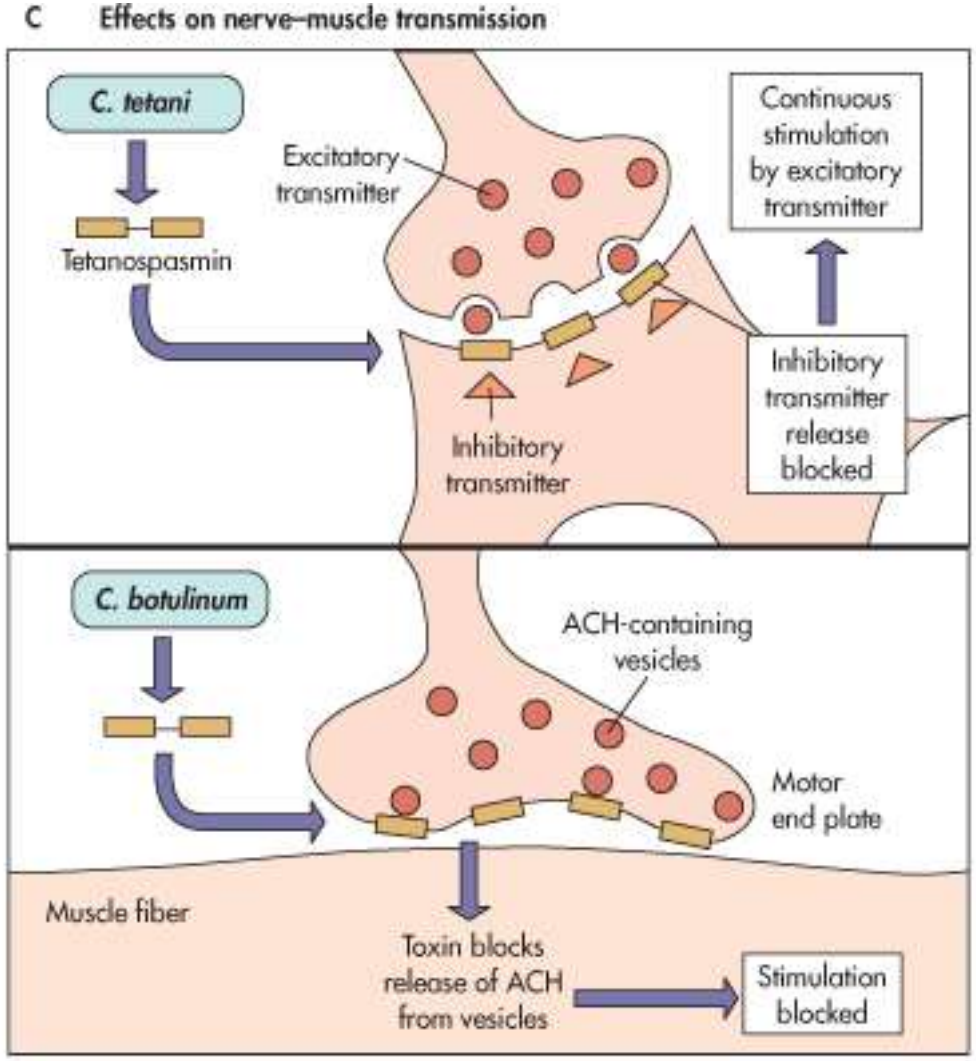
A child with tetanus and opistotonus resulting from persistent spasms of the back muscles



Clostridium botulinum

- Physiology and Structure **Gram-positive, spore-forming** rod, **strict anaerobe**, fastidious growth requirements, can produce one of seven distinct botulinum toxins (A to G)
- Virulence
  Spore formation, **botulinum toxin prevents release of neurotransmitter acetylcholine,** binary toxin
  Epidemiology
  Ubiquitous; C. botulinum spores are found in soil worldwide
  * Human diseases associated with toxins A, B, E, and F
  * **Infant botulism** more common than other forms
  Diagnosis Botulism confirmed by **isolating the organism** or **detecting the toxin in food** products or the **patient's feces or serum**
  Treatment, Prevention, and Control Treatment involves administration of **metronidazole** or **penicillin G**, **trivalent botulinum antitoxin**, and **ventilatory support**
  * Spore germination in foods prevented by maintaining food in an acid pH, by high sugar content (e.g., fruit preserves), or by storing the foods at 4°C or colder
  * **Toxin is heat-labile** and therefore can be destroyed by heating of food for 10 minutes at 60° to 100°C
  *** Infant botulism is associated with consumption of contaminated foods** (particularly honey). Infants younger than 1 year should not be given honey or foods containing it

Mechanism of *C. tetani* and *C. botulinum* neurotoxins



Clostridium difficile

Physiology and Structure **Gram-positive**, **spore-forming rods**
* **Strict anaerobe** (vegetative cells are extremely oxygen sensitive)

Virulence **enterotoxin (toxin A)** and a **cytotoxin (toxin B)**

Epidemiology The organism is ubiquitous
* **Colonizes the intestines of a small proportion of healthy individuals** (<5%)
* **Risk factor - exposure to antibiotics is associated** with **overgrowth of *C. difficile*** and subsequent disease (**dysmicrobia**, endogenous infection)
* **Spores can be detected in hospital** rooms of infected patients (particularly around beds and in the bathrooms)

Diagnosis *C. difficile* disease is confirmed by **detecting** the somatic **antigen** (GDH), **cytotoxin** or **enterotoxin** in the patient's feces (bed side test)

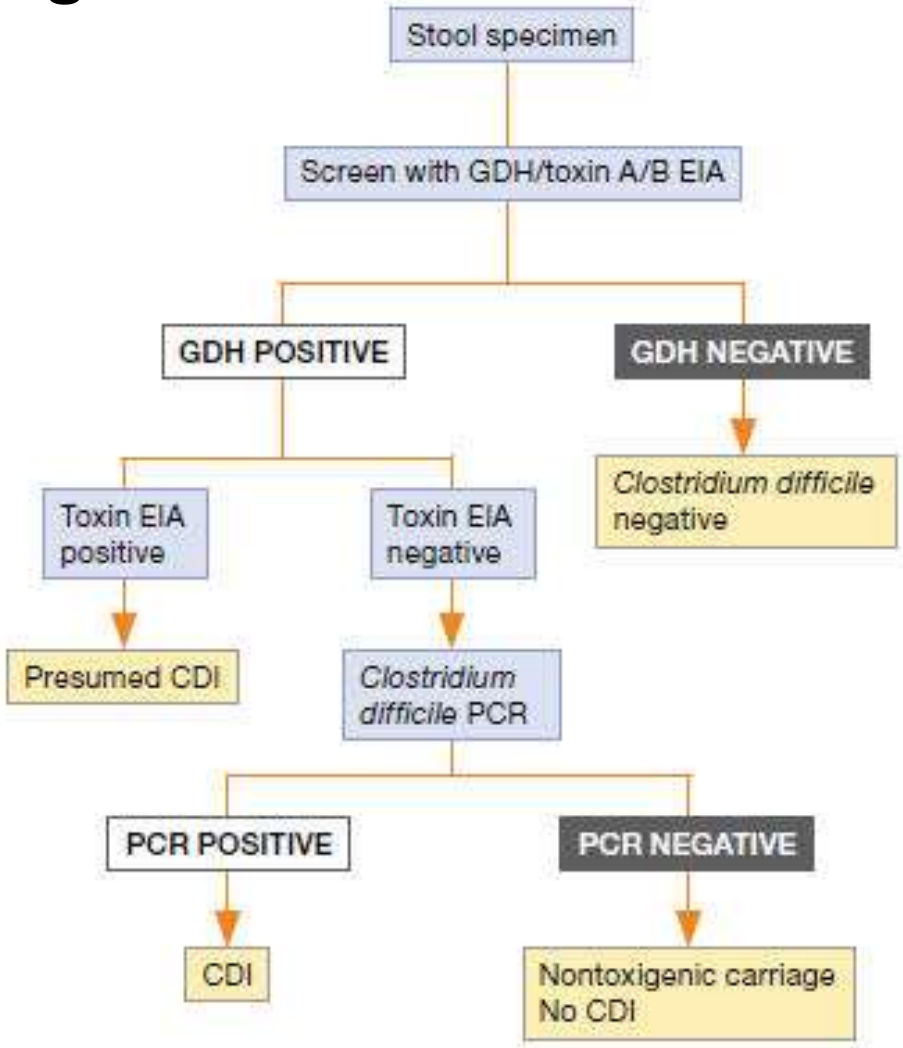
Treatment, Prevention, and Control **The implicated antibiotic should be discontinued**
* Treatment with **metronidazole** or **vancomycin** should be used in severe disease

• **Oral bacteriotherapy** - from family members

• Relapse is common because antibiotics do not kill spores; a second course of therapy with the same antibiotic is usually successful

• The hospital room should be carefully cleaned after the infected patient is discharged

Algorithm of C. difficile diagnosis



Somatic **antigen** (GDH), **cytotoxin** (toxin B) and **enterotoxin** (toxin A) in the patient's feces (bed side test)

Clostridium perfringens

Physiology and Structure

Large, **rectangular**, **gram-positive rods**, **subterminal spores** rarely seen in specimens or culture
Replicates rapidly, so large, spreading colonies are seen within **first day of culture**; **"double zone" of hemolysis** on blood agar (produced by α-and θ-toxins)

Virulence

Produces **many toxins and hemolytic enzymes**, so white blood cells and platelets are not seen in Gram-stained clinical specimens, lecithinase (phospholipase C)
Subdivided into five types (A to E) on the basis of toxin production

Epidemiology

Ubiquitous; present in soil, water, and **intestinal tract of humans and animals**
Type A is responsible for most human infections
Disease follows exogenous or endogenous exposure

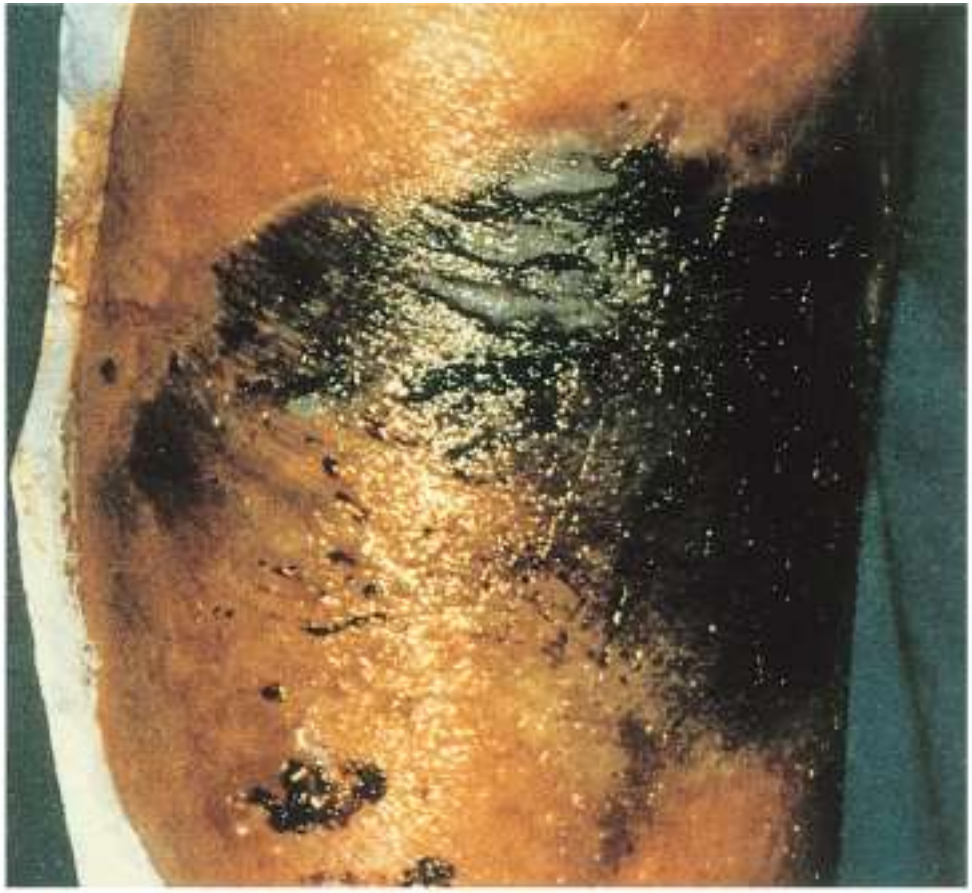
Diagnosis

Characteristic forms seen on Gram stain, grows rapidly in culture

Treatment, Prevention, and Control

Rapid treatment is essential for serious infections
Systemic infections require **surgical débridement and high-dose penicillin therapy; antiserum against α-toxin** not used today; the value **of hyperbaric oxygen** treatment is unproven
Symptomatic treatment for food poisoning (not ATB). **Proper wound care and judicious use of prophylactic antibiotics will prevent most infections.**

C. perfringens (introduced into tissue during surgery or trauma) cellulitis (subcutaneous gas present)



Clostridia - Clinical Summaries

Clostridium tetani

Generalized tetanus: Generalized musculature spasms and involvement of the autonomic nervous system in severe disease (e.g., cardiac arrhythmias, fluctuations in blood pressure, profound sweating, dehydration)

Localized tetanus: Musculature spasms restricted to localized area of primary infection

Neonatal tetanus: Neonatal infection primarily involving the umbilical stump; very high mortality

Clostridium botulinum

Foodborne botulism: Initial presentation of blurred vision, dry mouth, constipation, and abdominal pain; progresses to bilateral descending **weakness of the peripheral muscles with flaccid paralysis**

Infant botulism: Initially nonspecific symptoms (e.g., constipation, weak cry, failure to thrieve) that progress to **flaccid paralysis** and **respiratory arrest**

Wound botulism: Clinical presentation same as with foodborne disease, although the incubation period is longer and fewer gastrointestinal symptoms

Inhalation botulism: Inhalation exposure to botulinum toxin would be expected to have a rapid onset of symptoms (flaccid paralysis, pulmonary failure) and high mortality

Clostridia - Clinical Summaries

Clostridium perfringens

Soft-tissue infections

Cellulitis: localized edema and erythema with gas formation in the soft tissue; generally nonpainful

Suppurative myositis: accumulation of pus (suppuration) in the muscle planes without muscle necrosis or systemic symptoms

Myonecrosis: painful, rapid destruction of muscle tissue; systemic spread with high mortality

Gastroenteritis

Food poisoning: Rapid onset of abdominal cramps and watery diarrhea with no fever, nausea, or vomiting; short, self-limited duration

Necrotizing enteritis: Acute, necrotizing destruction of jejunum with abdominal pain, vomiting, bloody diarrhea, and peritonitis

Clostridium difficile

Antibiotic-associated diarrhea: Acute diarrhea generally developing 5 to 10 days after initiation of antibiotic treatment (particularly clindamycin, penicillins, and cephalosporins); may be brief and self-limited or more protracted

Pseudomembranous colitis: Most severe form of *C. difficile* disease with profuse diarrhea, abdominal cramping, and fever; whitish plaques (pseudomembranes) over intact colonic tissue seen on colonoscopy. **Toxic megacolon: an acute form of colonic distension characterized by a dilated colon (megacolon), accompanied by**

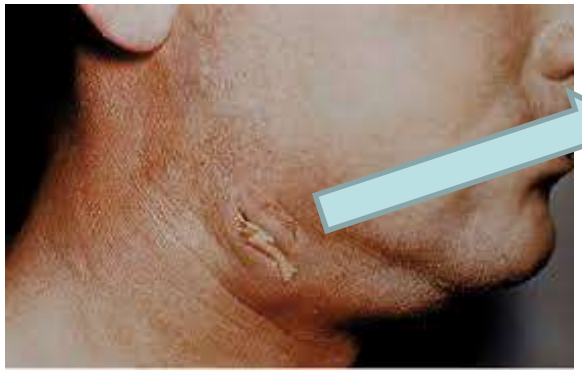
Non-sporeforming anaerobic bacteria

Grampositive cocci: ***Peptostreptococcus, Peptococcus spp.*** – **inhabitant of oral cavity, GIT, skin,** infection – when they spread from the normal sites (e.g. from upper respiratory tract to sinuses – **sinusitis**, from genitourinary tract – **endometritis, pelvic abscesses)**, **therapy** – **usually susceptible to penicillin**

Grampositive rods: ***Actinomyces spp.*** **–** *e.g.* ***A. israelii,*** **inhabits mucosal surfaces –upper respiratory tract and female genitourinary tract**, **grow slowly** (cca 2 weeks) on culture media, microscopy **gram-positive rods and filaments**, low virulence – causes **endogenous disease** only when normal mucosal barriers are disrupted by trauma or surgery. **Clinical infections** – **cervicofacial actinomycosis** (after poor oral hygiene, after invasive dental procedures), thoracic actinomycosis (after aspiration), **abdominal and pelvic inf**. after **GIT surgery** or as **primary inf. n women with intrauterine device**, **treatment** – tissue debridement and penicillin.

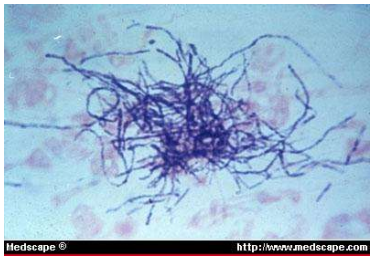
Other grampositive rods: ***Propionibacterium acnes*** – after digestion by PMN in sebaceous glands enzymes like lipases are released and cause inflammation in teenagers, can causes also opportunistic infections (e.g. infection of artifical heart valves). ***Lactobacillus*** **spp**. – part of normal flora of the mouth, stomach, intestines, and genitourinary tract (vagina – normal flora with probiotic effect, lowering pH)

Diagnosis of actinomycosis



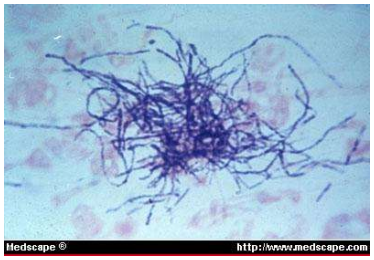
Dermatologist.com

Sample collection: pus



Microscopy preparation, Gram-positive rods and filaments
PCR detection

Culture after 14days of cultivation



Microscopy prepation, Gram-positive rods and filaments

Identification (mass spectrometry)

Identification by PCR

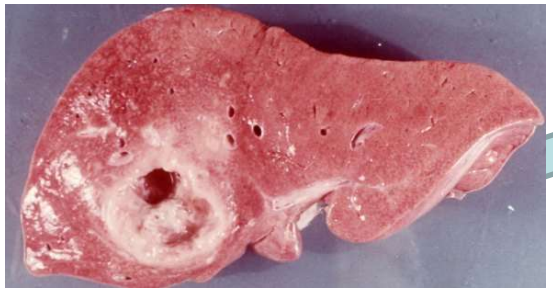
Diagnosis: cervicofacial actinomyosis (lesion – actinomycetoma)

Non-sporeforming anaerobic bacteria

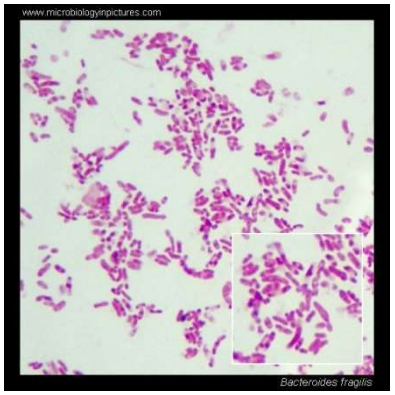
Gram-negative cocci: ***Veilonella*** **spp.**
Gram-negative rods: ***Bacteroides*** **spp.(50 species)** – LPS lower endotoxic activity**,** ***Fusobacterium*** **spp.,** ***Prevotella*** **spp.**

• Colonize the **upper respiratory tract, GIT, genitourinary tract**
• Most significant pathogen - ***Bacteroides fragilis*** **, resides in GIT**, causing **abdominal infections** (e.g. liver absceses) and bacteremia after disruption of natural barriers by diagnostic or surgical procedures, **pleomorphic gram-negative rods**, capsule – adhesive and antiphagocytic properties, **laboratory diagnosis** – culture, treatment – usually produce beta-lactamases, **drug of choice – metronidazole, carbapenems**.

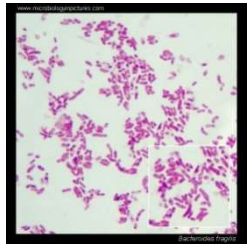
Diagnosis of liver abscess infection



Sample collection: pus



Microscopy preparation, negative pleomorphic rods
PCR detection



Microscopy preparation, negative pleomorphic rods

Culture

Identification (mass spectrometry)

Identification by PCR

Diagnosis: liver abscess caused by *B. fragilis*

The place of molecular genetic methods in the diagnostics of anaerobic bacteria (Nagy et al., 2006, Acta Microbiol Immunol Hung.)

- diverse group of bacteria
- specimens yielding anaerobic bacteria commonly contain several organisms and often very complex mixtures of aerobic and anaerobic bacteria, considerable time may elapse before the final report.
- Species definition based on phenotypic features is often time-consuming and is not always easy to carry out.
- Molecular genetic methods may help in the everyday clinical microbiological practice (such as 16S rRNA PCR-RFLP profile determination), which can help to distinguish species
- Some anaerobic bacteria are extremely slow growing or not cultivable at all but detectable by molecular methods which also demonstrated the spread of specific resistance genes among the most important anaerobic bacteria.
- Molecular methods (a search for toxin genes and ribotyping) may promote a better understanding of the pathogenic features of some anaerobic infections, such as the nosocomial diarrhoea caused by *C. difficile* and its spread in the hospital environment and the community.

Diagnosis of anaerobic infections

Clinical signs include:

- foul-smelling discharge (becuase of the end product of anaerobic metabolism – short-chain fatty-acids)
- infection in proximity to a mucosal surface (anaerobes are part of the normal flora)
- gas in tissue (production of CO_2 and H_2)
- negative aerobic cultures