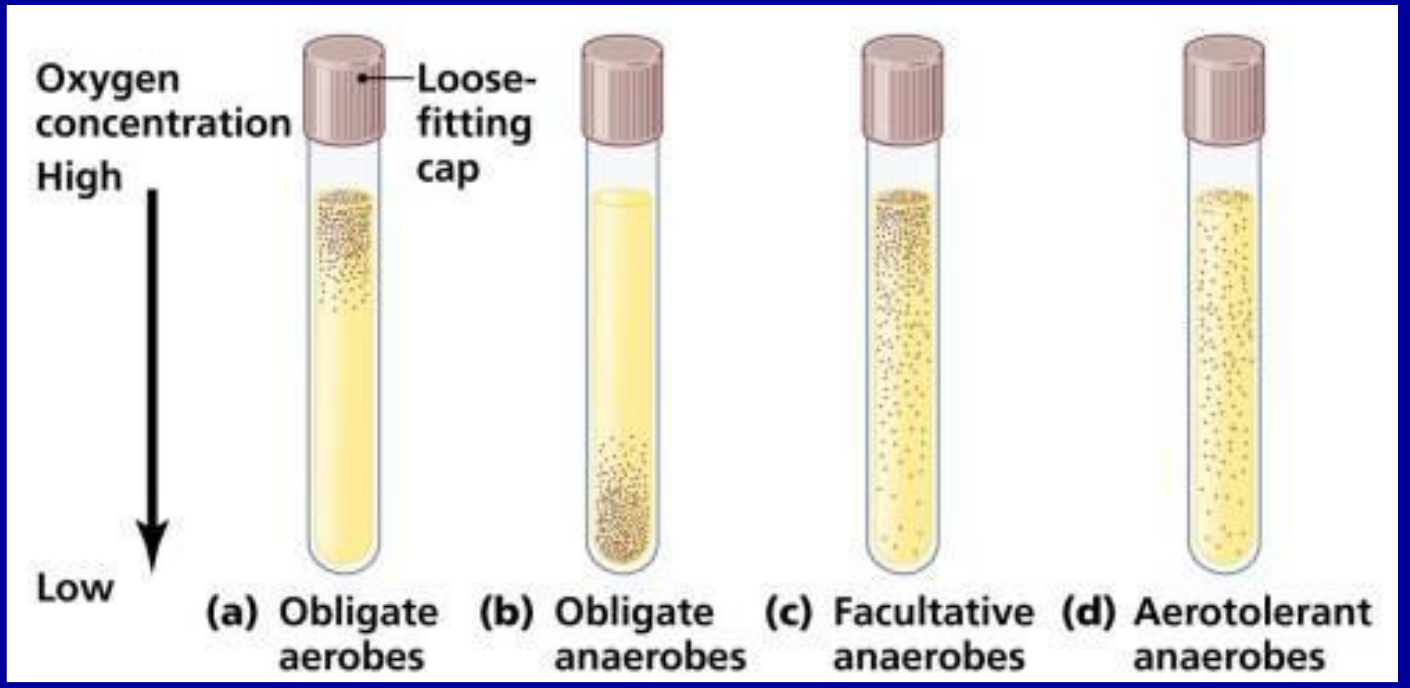
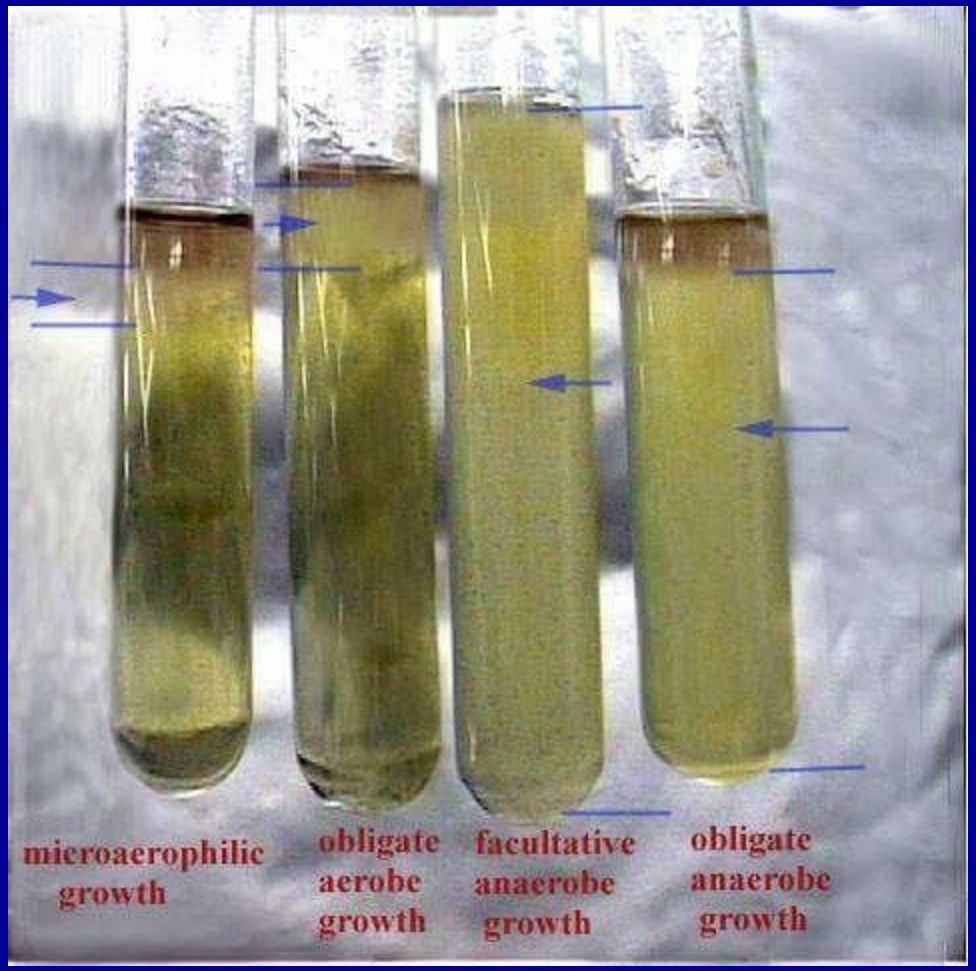
Anaerobes

Cagla BOZKURT-GUZEL


Oxygen concentration
High
Low
Loose-fitting cap
(a) Obligate aerobes
(b) Obligate anaerobes
(c) Facultative anaerobes
(d) Aerotolerant anaerobes


microaerophilic growth
obligate aerobe growth
facultative anaerobe growth
obligate anaerobe growth

Definitions

- Anaerobes
  - Bacteria that require anaerobic conditions to initiate and sustain growth
- Strict (obligate) anaerobe
  - Unable to grow if > than 0.5% oxygen (Clostridium)
- Moderate anaerobes
  - Capable of growing between 2-8% oxygen (*Haemophilus influenzae, Neisseria gonorrhoea*)
- Microaerophillic bacteria
  - Grows poorly in air, but better in anaerobic conditions (*Camphylobacter jejuni, Helicobacter pylorii*)
- Facultative bacteria (facultative anaerobes)
  - Grows both in presence and absence of air (*S. aureus*)

Classification of Medically Important Anaerobes

- **Gram positive cocci**
  - Peptostreptococcus
- **Gram negative cocci**
  - Veillonella
- **Gram positive bacilli**
  - *Clostridium perfringens, tetani, botulinum, difficile*
  - Propionibacterium
  - Actinomyces
  - Lactobacillus
  - Mobiluncus
- **Gram negative bacilli**
  - *Bacteroides fragilis, thetaiotaomicron*
  - Fusobacterium
  - Prevotella
  - Porphyromonas

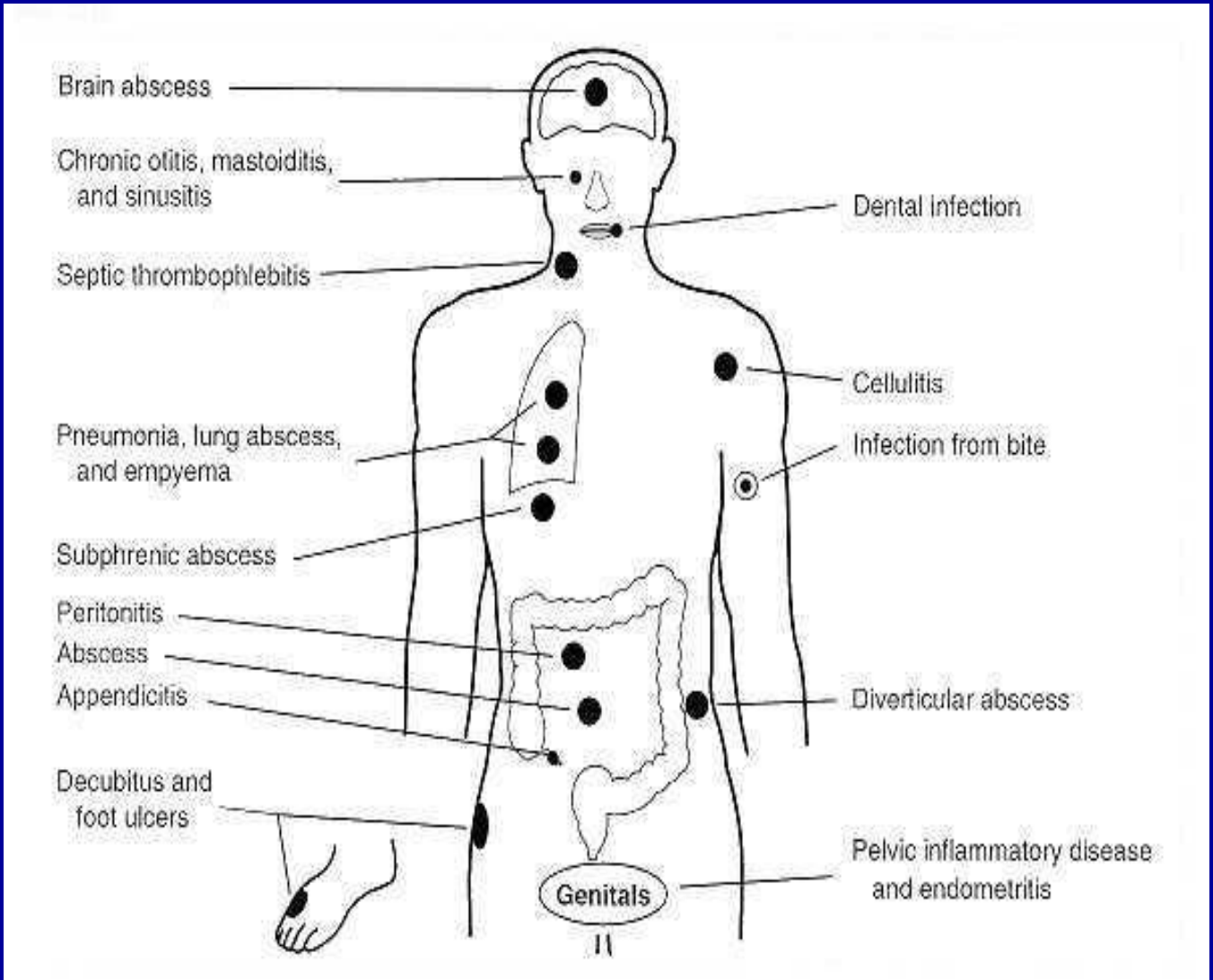
Epidemiology

- Endogenous infections
  - Indigenous microflora
    - Skin: Propionibacterium, Peptostreptococcus
      - Prevalence in areas exposed to air explained by (1) oxygen consumption by aerobes (2) low oxidation-reduction potential microhabitats
    - Upper respiratory: Propionibacterium
    - Mouth: Fusobacterium, Actinomyces
    - Intestines: Clostridium, Bacteroides, Fusobacterium
    - Vagina: Lactobacillus
  - Profound modification of flora in pathophysiologic states
    - Antimicrobials and other medications (PPI, antacids)
    - Surgery (blind loops)
    - Cancers
- Exogenous infections

Role of Anaerobes

- Role in normal host physiology
  - Prevent colonization & infection by pathogens
    - Bacterial interference through elaboration of toxic metabolites, low pH, depletion of nutrients
    - Interference with adhesion
  - Contributes to host physiology
    - *B. fragilis* synthesizes vitamin K and deconjugates bile acids

Sites of anaerobic infections



Virulence factors

- Attachment and adhesion
  - Polysaccharide capsules and pili
- Invasion
  - Alteration in host tissue (trauma, disease)
  - Aerotolerance
- Establishment of infection
  - Polysaccharide capsule (*B. fragilis*)
  - Spore formation (Clostridium)
  - Maintenance of reduced environment
- Tissue damage
  - Elaboration of toxins

Clinical features of anaerobic infections

- The source of infecting micro-organism is the endogenous flora of host
- Alterations of host's tissues provide suitable conditions for development of opportunist anaerobic infections
- Anaerobic infections are generally polymicrobial
- Abscess formation
- Exotoxin formation

TABLE 20-2 Conditions Predisposing to Anaerobic Infection

- General
  - Diabetes
  - Corticosteroids
  - Leukopenia
  - Hypogammaglobulinemia
  - Immunosuppression
  - Cytotoxic drugs
  - Splenectomy
  - Collagen disease
- Decreased redox potential
  - Tissue anoxia
  - Tissue destruction
  - Aerobic infection
  - Foreign body
  - Calcium salts
  - Burns
  - Peripheral vascular insufficiency
- Specific clinical situations
  - Cancer
    - Colon, uterus, lung
    - Leukemia
  - Gastrointestinal and female pelvic surgery
  - Gastrointestinal trauma
  - Human and animal bites
  - Aminoglycoside therapy

Anaerobic cocci

- Epidemiology
  - Normal flora of skin, mouth, intestinal and genitourinary tracts
- Pathogenesis
  - Opportunistic pathogens, often involved in polymicrobial infections
  - Virulence factors not as well characterized
  - Brain abscesses, periodontal disease, pneumonias, skin and soft tissue infections, intra-abdominal infections
- Peptostreptococcus
  - P. *magnus*: chronic bone and joint infections, especially prosthetic joints
  - P. *prevotti* and P. *anaerobius*: female genital tract and intra-abdominal infections
- Veillonella
  - Normal oral flora; isolated from infected human bites

Anaerobic gram positive bacilli

- No Spore Formation
  - Propionibacterium
    - P. *acnes*
  - Actinomyces
    - A. *israelii*
  - Lactobacillus
  - Mobiluncus
- Spore Formation
  - Clostridium
    - C. *perfringens*
    - C. *difficile*
    - C. *tetani*
    - C. *botulinum*

Propionobacterium

- Anaerobic or aerotolerant, produces propionic acid as major byproduct of fermentation
- Colonize skin, conjunctiva, external ear, oropharynx, female GU tract
- *P. acnes*
  - Acne
    - Resides in sebaceous follicles, releases LMW peptide, stimulates an inflammatory response
  - Opportunistic infections
    - Prosthetic devices (heart valves, CSF shunts)

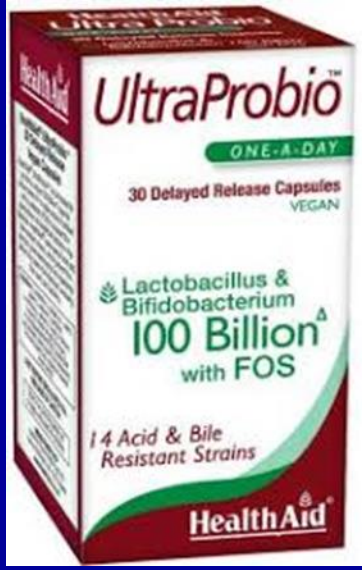
Actinomyces

- Facultative or strict anaerobes
- Colonize upper respiratory tract, GI, female GU tract
- Low virulence; development of disease when normal mucosal barriers are disrupted
- Diagnosis:
  - Macroscopic colonies of organisms resembling grains of sand (sulfur granules)
  - Culture

Actinomycosis

- Cervicofacial Actinomycosis
  - Poor oral hygiene, oral trauma
  - Slowly evolving, painless process
  - Chronic granulomatous lesions that become suppurative and form sinus tracts
  - Treatment: surgical debridement and prolonged penicillin

Lactobacillus



- Facultative or strict anaerobes
- Colonize GI and GU tract
  - Produces H_2O_2 which is bactericidal to *Gardnerella vaginalis*
  - Vagina heavily colonized (10^5/ml) by Lactobacillus *crispatus & jensonii*
- Clinical disease
  - Transient bacteremia from GU source
  - Endocarditis
  - Bacteremia in immunocompromized host

Mobiluncus

- Obligate anaerobes
- Gram negative or gram variable
- Colonize GU tract in low numbers
- Associated with bacterial vaginosis
  - Detected in vagina of 6% of controls
  - As many as 97% of women with bacterial vaginosis

Clostridium

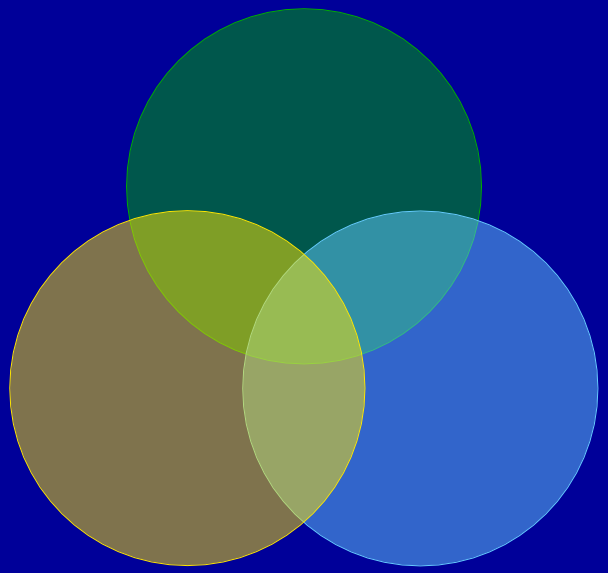
- Epidemiology
  - Ubiquitous,
    - Present in soil, water, sewage
    - Normal flora in GI tracts of animals and humans
- Pathogenesis
  - Spore formation
    - resistant to heat, dessication, and disinfectants
    - can survive for years in adverse environments
  - Rapid growth in nutritionally enriched, oxygen deprived environment
  - Toxin elaboration (histolytic toxins, enterotoxins, neurotoxins)

Clostridium perfringens

- Epidemiology
  - GI tract of humans and animals
  - Type A responsible for most human infections
- Pathogenesis
  - α-toxin: lecithinase (phospholipase C) that lyses erythrocytes, platelets and endothelial cells
  - ß-toxin: necrotizing activity
  - θ-toxin: hemolysin
  - Enterotoxin: binds to brush borders and disrupts small intestinal transport
- Clinical manifestations
  - Self-limited gastroenteritis
  - Soft tissue infections: cellulitis, fascitis or Myonecrosis (gas gangrene)

Clostridial soft tissue infections

Crepitant cellulitis



Fascitis

Myonecrosis

Myonecrosis

Clean wound

Gangrenous wound

Fig. 84 Gas gangrene.

Myonecrosis xray

Clostridium difficile

- Epidemiology
  - Colonizes GI tract of 5% healthy individuals
  - Endogenous infection
    - antibiotic exposure associated with overgrowth of C. *difficile*
  - Exogenous infection
    - spores detected in hospital rooms of infected patients
- Pathogenesis
  - Enterotoxin (toxin A)
    - produces chemotaxis, induces cytokine production and hypersecretion of fluid, development of hemorrhagic necrosis
  - Cytotoxin (toxin B)
    - Induces polymerization of actin with loss of cellular cytoskeleton

C. difficile colitis

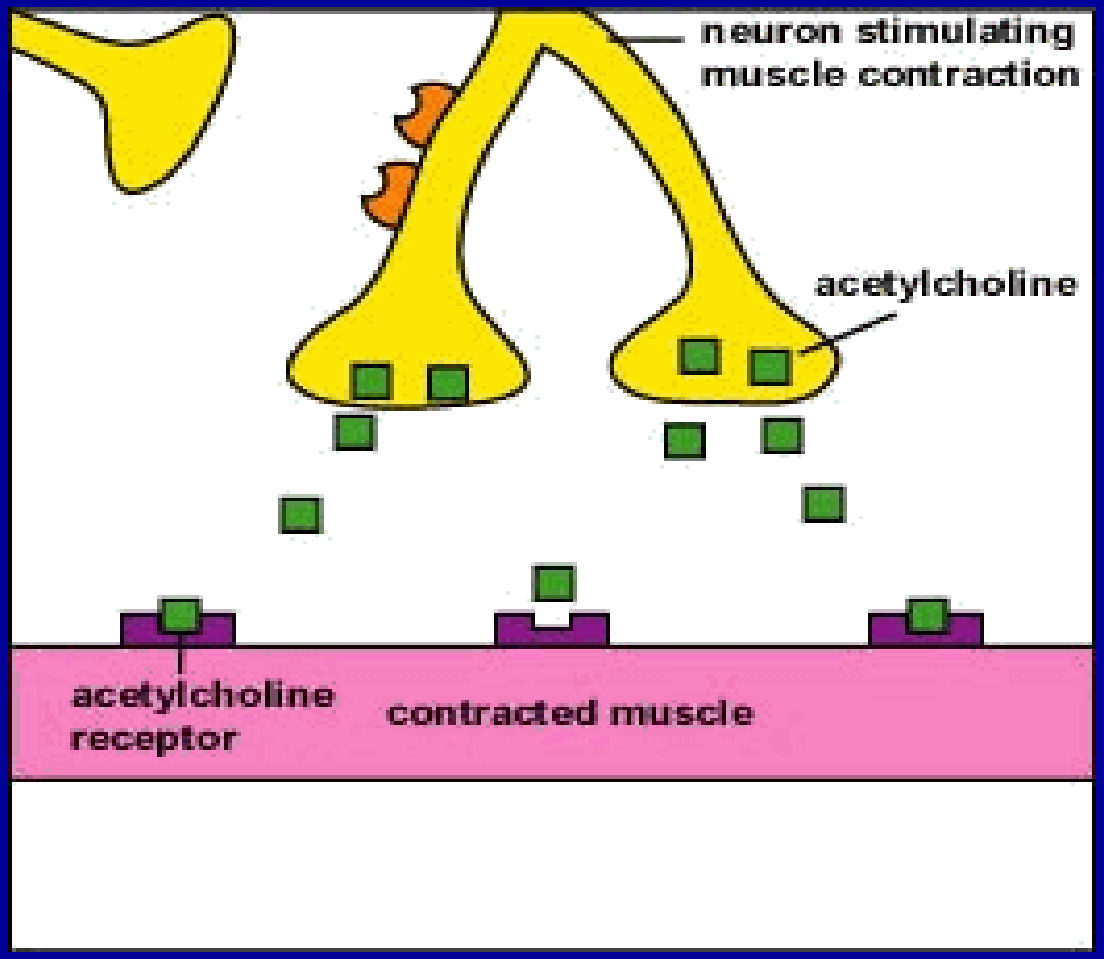
C. *difficile* colitis

- Clinical syndromes
  - Asymptomatic colonization
  - Antibiotic-associated diarrhea
  - Pseudomembranous colitis
- Diagnosis
  - Isolation of cytotoxin or enterotoxin
- Treatment
  - Discontinue antibiotics
  - Metronidazole or vancomycin
  - Relapse in 20-30% (spores are resistant)

Clostridium tetani

- Epidemiology
  - Spores found in most soils
  - Disease in un-vaccinated or inadequately immunized
  - Disease does not induce immunity
- Pathogenesis
  - Spore inoculated into wound
  - Tetanospasmin
    - Heat-labile neurotoxin
    - Retrograde axonal transport to CNS
    - Blocks release of inhibitory neurotransmitters (GABA) resulting in spastic paralysis
    - Binding is irreversible
  - Tetanolysin
    - Oxygen labile hemolysin, unclear clinical significance

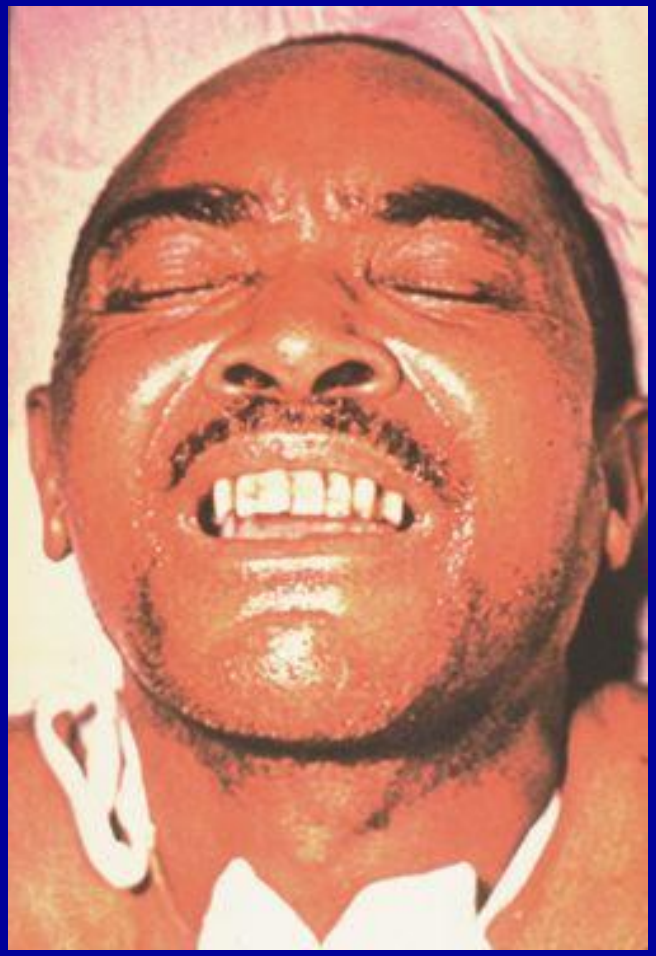
C. tetani exotoxin



Tetanus

- Clinical Manifestations
  - Generalized
    - Involvement of bulbar and paraspinal muscles
      - Trismus, risus sardonicus, opisthotonos
    - Autonomic involvement
      - Sweating, hyperthermia, cardiac arrythmias, labile BP
  - Cephalic
    - Involvement of cranial nerves only
  - Localized
    - Involvement of muscles in primary are of injury
  - Neonatal
    - Generalized in neonates; infected umbilical stump

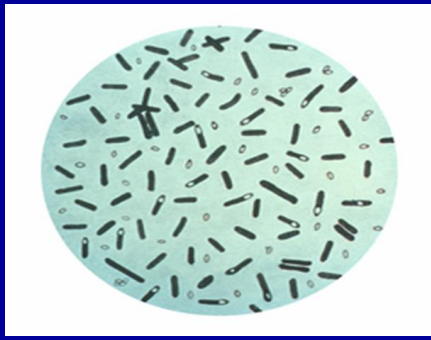
Risus sardonicus and Opisthotonos of Tetanus



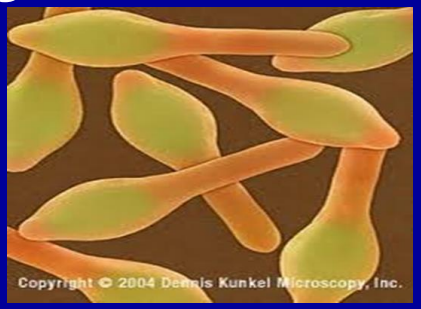
Tetanus

- Treatment
  - Debridement of wound
  - Metronidazole
  - Tetanus immunoglobulin
  - Vaccination with tetanus toxoid
- Prevention
  - Vaccination with a series of 3 tetanus toxoid
  - Booster dose every 10 years

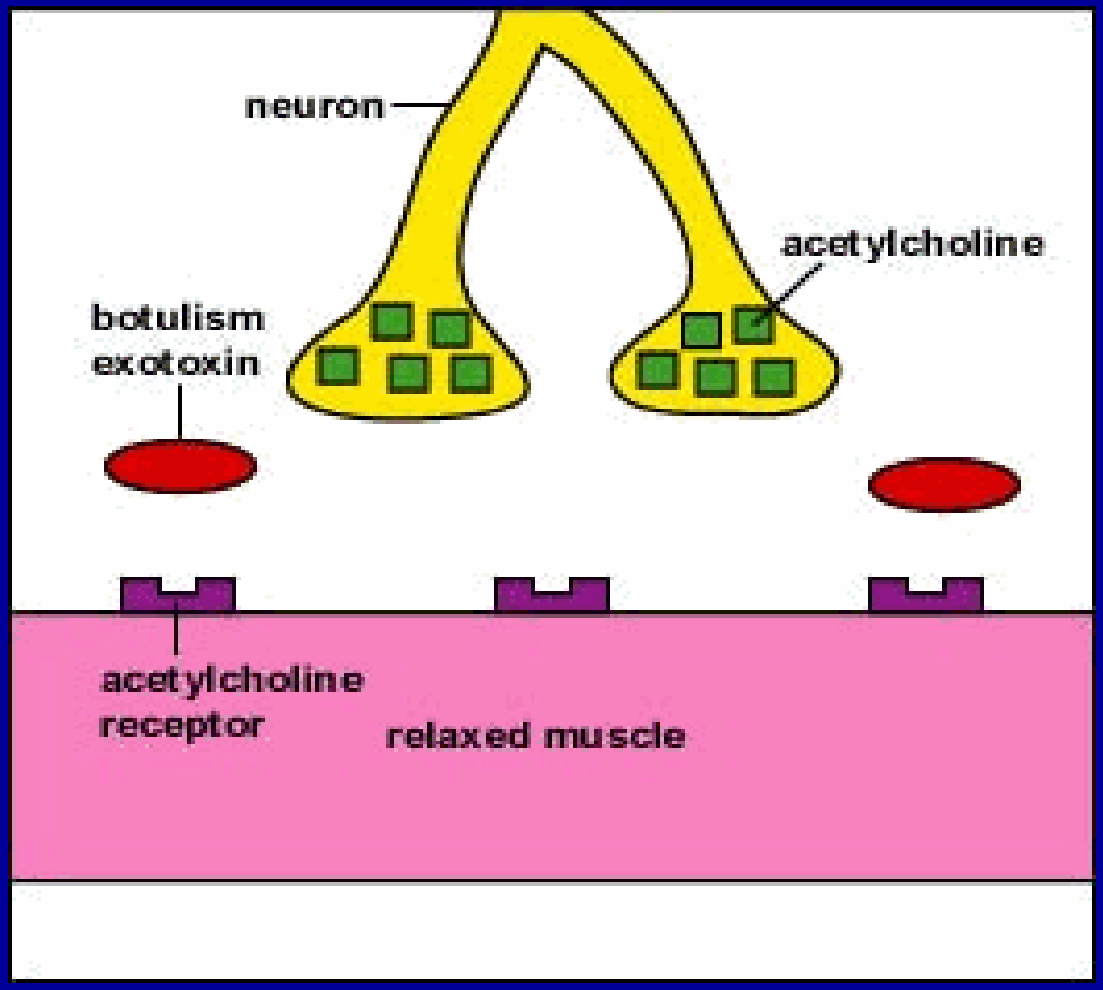
Clostridium *botulinum*



- Epidemiology
  - Commonly isolated in soil and water
  - Human disease associated with A, B, E, F
- Pathogenesis
  - Botulinum toxin targets cholinergic nerves
  - Prevents release of acetylcholine
  - Recovery depends upon regeneration of nerve endings



C. Botulinum Exotoxin



Botulism

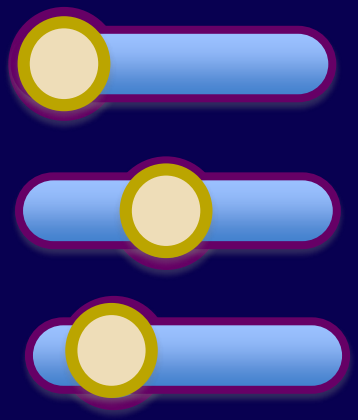
- Clinical Syndromes
  - Foodborne botulism
    - Mostly associated with home-canned foods and preformed toxin
    - Onset of symptoms 1-2 days: blurred vision, dilated pupils, dry mouth, constipation
    - Bilateral descending weakness of peripheral muscles; death related to respiratory failure
  - Infant botulism
    - Consumption of foods contaminated with botulinum spores
    - Disease associated with neurotoxin produced in vivo
  - Wound botulism

Botulism

- Diagnosis
  - Isolation of organism
    - Culture implicated food and stool of patient
  - Isolation of toxin
    - Mouse bioassay
- Treatment
  - Supportive care
  - Elimination of organism from GI tract
    - Gastric lavage
    - Metronidazole or penicillin
  - Trivalent botulinum toxin (A, B, E) to bind circulating botulinum toxin
- Prevention
  - Prevention of spore germination (Acid PH, storage <4°C)
  - Destruction of preformed toxin (20 min at 80°C)

Spores

Spore positioning and species



Terminal spore	*C. tetani*
Central spore	*C. perfringens*
Subterminal spore	*C. septicum, novyi, histolyticum difficile, botulinum*

Anaerobic gram negative bacilli

- Bacteroides
  - *B. fragilis*
  - *B. thetaiotaomicron*
- Fusobacterium
- Prevotella
- Porphyromonas

Anaerobic gram negative bacilli

- Epidemiology
  - Colonize human body in great numbers
    - Stabilize resident bacterial flora
    - Prevent colonization by pathogens
  - Anaerobes are predominant bacteria in upper respiratory tract, GI and GU tract
    - Outnumber aerobic bacteria by 10-100 fold
    - Many species, but few pathogens

Anaerobic gram negative bacilli

- Clinical Diseases
  - Chronic sinus infections
  - Periodontal infections
  - Brain abscess
  - Intra-abdominal infection
  - Gynecological infection
  - Skin and soft tissue

Bacteroides

- Epidemiology
  - *B.* fragilis associated with 80% of intra-abd infx
- Pathogenesis
  - Polysaccharide capsule
    - Increases adhesion to peritoneal surfaces (along with fimbriae)
    - Protection against phagocytosis
    - Differs from LPS of aerobic GNR
      - Less fatty acids linked to Lipid A component
      - Less pyrogenic activity
  - Superoxide dismutase and catalase
  - Elaborate a variety of enzymes

Bacteroides

- Infections
  - Intra-abdominal infections (peritonitis, abscess); bacteremias; decubitus and diabetic ulcers
- Treatment
  - Drainage of abscess and debridement of necrotic tissue
  - Antibiotics

ANAEROBIC BACTERIA - CULTIVATION

- Thioglycolate broth is a multi-purpose, enriched differential medium used primarily to determine the oxygen requirements of microorganisms.

Special culture techniques for anaerobic bacteria

- Candle jar

A candle jar is a container into which a lit candle is introduced before sealing the container's airtight lid . The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar.

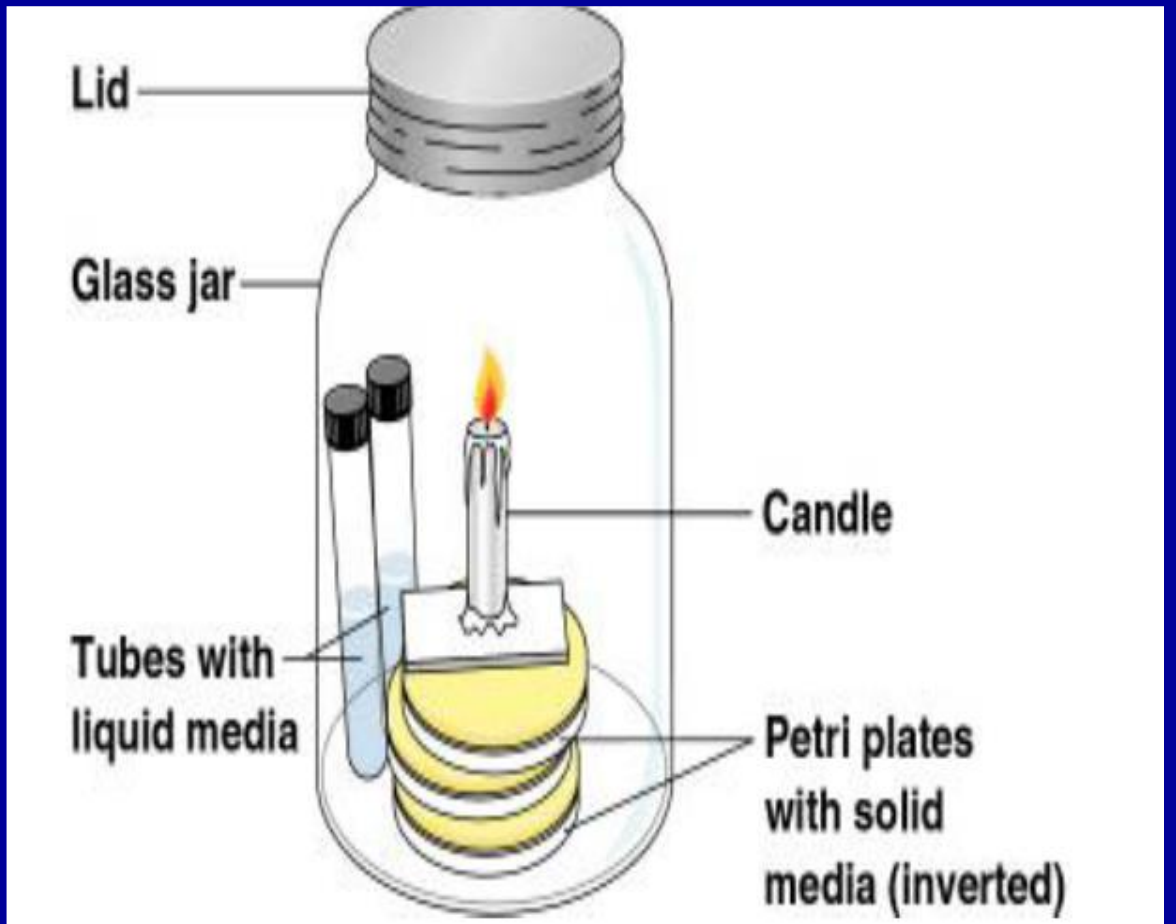


Fig. 15 Candle jar.

Gas pack

- Gas packs can generate CO_2 also and are generally used in place of candle jars.
- The packet consist of a bag containing a Petri plate and CO_2 gas generator.
- The gas generator is crushed to mix the chemicals it contains and start the reaction that produces CO_2.
- This gas reduces the oxygen concentration in the bag to about 5% and provides CO_2 concentration of about 10%.

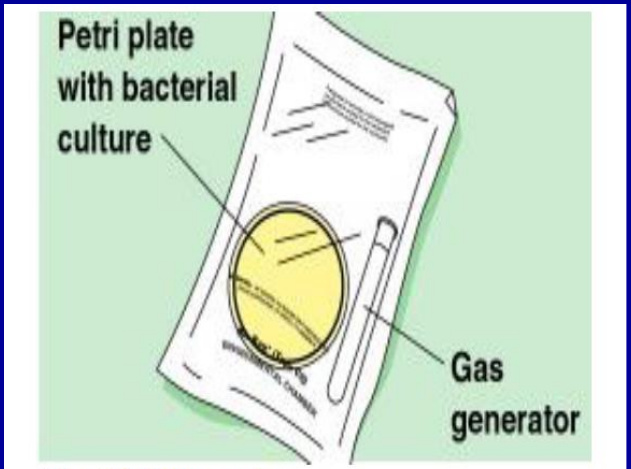


Fig. 16 Gas pack.

ANAEROBIC JAR

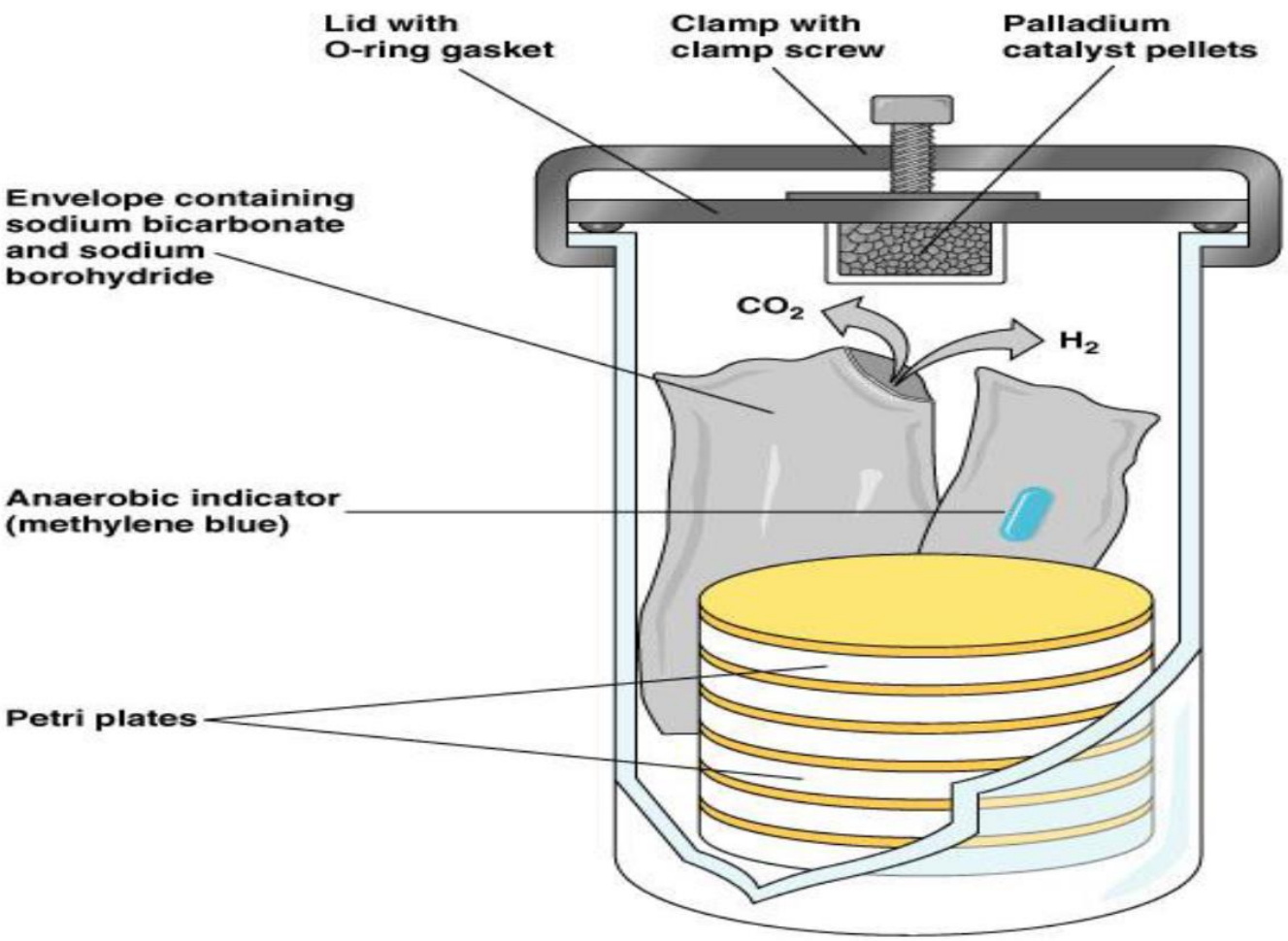


Fig. 17 Anaerobic jar.

- Petri plates can be incubated in an anaerobic jar or anaerobic chamber.
- Sodium bicarbonate and sodium borohydride are mixed with a small amount of water to produce CO_2 and H+.
- A palladium catalyst in the jar combines with the O_2 in the jar and the H+ to remove O_2.