

- A 50 year old diabetic patient presented with a ulcer, swelling and pain of the lower limb and fever for 5 days .
- On examination the limb was extremely tender, red and oedematous. There was necrotic areas in the wound with underlying crepitus, and foul smelling pus discharge from the wound.
- Patient looked ill with high fever and tachycardia and slightly low BP.
 - Probable diagnosis?
 - Aetiologies?
 - Specimens
 - Diagnostic methods?
 - Management?

Anaerobic infections

Dr Nadisha Badanasinghe

Objectives

- Anaerobic pathogens
 - Characteristics
 - Habitat
- Anaerobic Infections
 - Risk factors
 - Transmission
- Specimens for diagnosis
- Transport
- Diagnostic methods
- Management

Definitions

- OBLIGAETE ANAEROBE
 - Grow ONLY in the absence of molecular oxygen
 - Killed by exposure to air
- AERO-TOLERANT ANAEROBES
 - survive in presence of oxygen
 - Can tolerate exposure to air for several hours
 - Do not use oxygen for energy requirements/ growth
- FACULTATIVE ANAEROBES
 - Do not require O₂, but use it if available
- MICROAEROPHILIC
 - that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere

Obligate anaerobes

- Lack certain enzymes
 - superoxide dismutase
$$\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2$$
 - catalase
 - $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$
 - peroxidase
 - $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} / \text{NAD to NADH}$
- Toxic radicals formed when exposed to $\text{O}_2 \rightarrow$ kill organisms

Anaerobes

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graph TD; Anaerobes --> Non_sporing[Non sporing]; Anaerobes --> Spore_forming[Spore forming]; Non_sporing --> Gram_neg[Gram neg]; Non_sporing --> Gram_pos[Gram Pos]; Spore_forming --> Clostridia[Clostridia]; Gram_pos --> Cocci[Cocci]; Gram_pos --> Bacilli[Bacilli]; Gram_neg --> Gram_neg_list["• Bacteroides<br/>• Leptotrichia<br/>• Fusobacterium"]; Cocci --> Peptostreptococcus[Peptostreptococcus]; Bacilli --> Lactobacillus[Lactobacillus]; Bacilli --> Propionibacterium[Propionibacterium]; Bacilli --> Actinomyces[Actinomyces];
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Non sporing

Spore forming

Gram neg

- Bacteroides
- Leptotrichia
- Fusobacterium

Gram Pos

Clostridia

Cocci

Bacilli

Peptostreptococcus

Lactobacillus

Propionibacterium

Actinomyces

Classification of Non - sporing Anaerobes

Gram -ve bacilli	Gram +ve bacilli
Bacteroides Prevotella Porphyromonas Fusobacterium Leptotrichia	Eubacterium Propionibacterium Lactobacillus Mobiluncus Bifidobacterium Actinomyces
Gram +ve Cocci	Gram -ve Cocci
Peptococci Peptostreptococci	Veillonella

Habitat

- Found in soil, fresh and salt water, and in normal flora of humans and animals
- Anaerobes that live outside the body are called “exogenous anaerobes”
(Example: Clostridium species)
- Anaerobes that live inside the body are called “endogenous anaerobes”
 - Advantages: barrier to colonization by pathogenic organisms, source of fatty acids, vitamins and cofactors, help mature neonate immune system
 - Disadvantages: opportunists for immunocompromised
- **Most anaerobic infections are from endogenous sources**
 - **Exceptions: Clostridial infections, animal/ human bite infections**

Virulence Factors

1. Anti-phagocytic capsule
 - Also promote abscess formation
2. Tissue destructive enzymes
 - *B. fragilis* produces variety of enzymes (lipases, proteases, collagenases) that destroy tissue → Abscess Formation
3. Beta-lactamase production
 - *B. fragilis* – protect themselves and other species in mixed infections
4. Adherence factors

Endogenous anaerobes

- Mucosal surfaces such as linings of oral cavity, upper respiratory tract, GI tract, female genital tract and skin
 - >90% of oral flora are anaerobes
 - cervical and vaginal areas have 50% anaerobes
 - 2/3's of all bacteria in the stool are anaerobes

Factors that Predispose Patients for Anaerobic Infections

- Trauma to mucosal membranes or skin
- Interruption of blood flow
- Tissue necrosis
- Decrease in redox potential in tissues
- Concomitant aerobic/ facultative anaerobic infections
- Prior antibiotic therapy when organism was resistant
- Immunosuppression
- Translocation to sites other than normal habitat

Endogenous Anaerobic Infections

- Oral flora → oral, head, neck, resp. infections
- GIT flora → Abdominal, pelvic infections
- Genital flora → genital tract (endometrium, tubes), BV
- Frequently cause mixed / polymicrobial infections
 - mixtures of Gram —ve rods (e.g. Bacteroides, Prevotella and Fusobacterium species) and Gram+ve cocci (peptostreptococci or streptococci or both)

Indications of Anaerobic Infections

- Usually purulent (large amount of pus)
- Close proximity to a mucosal surface
- Infection persists despite antibiotic therapy
- Presence of foul odor
- Presence of large quantities of gas (bubbling or cracking sound when tissue is pushed)
- Infection associated with necrotic tissues
- Septic thrombophlebitis
- Detection of “sulphur granules” in pus (actinomycosis)
- Deep abscess
- Gram negative septicaemia

Gram negative Bacilli

Bacteroidaceae

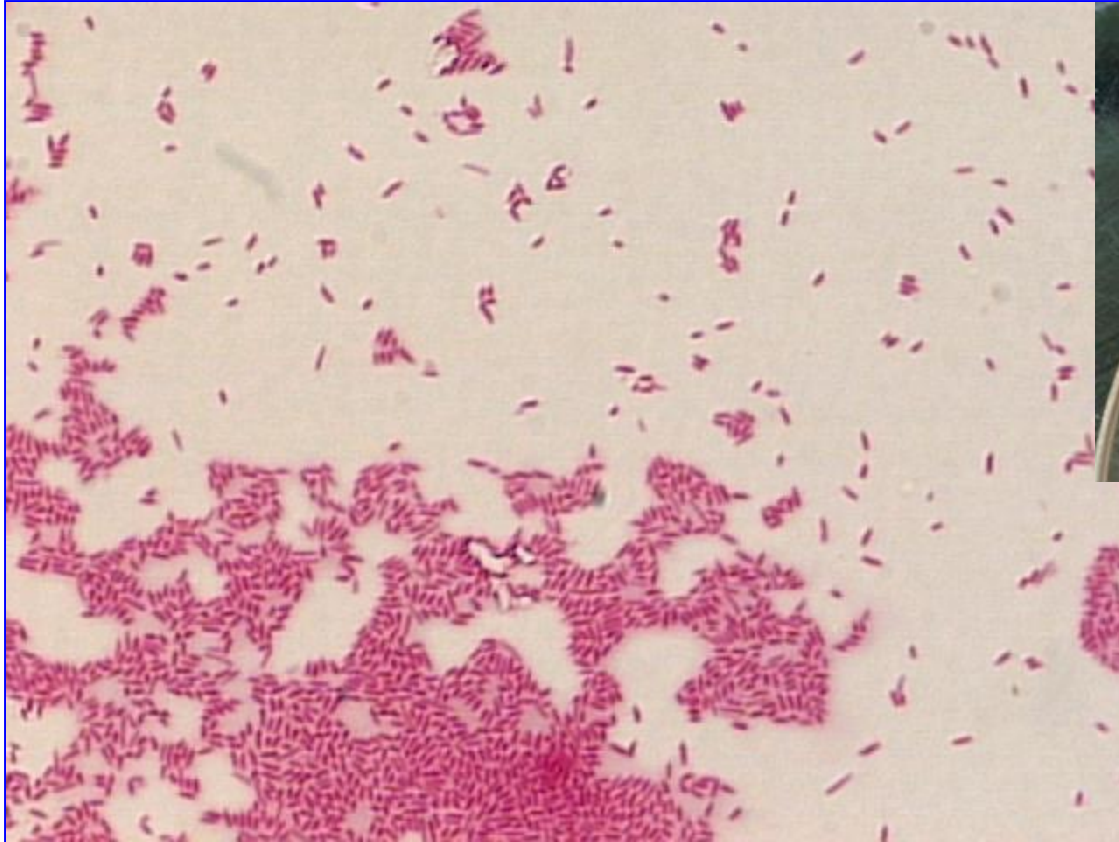
- Classification
 - Bacteroidaceae family includes the following genera
 - *Bacteroides*
 - *Fusobacterium*
 - *Leptotrichia* (rare in human diseases)
 - *Prevotella*
 - *Porphyromonas*
 - *Bacteroides*- Most common anaerobes isolated from clinical specimens

Gram negative bacilli

- Bacteroides
 - *B. fragilis* is the most commonly isolated anaerobe
 - Pleomorphic, capsulated, no pigments
 - Beta lactamase producers- Resistant to penicillin
 - Infections below umbilicus
- Prevotella & Porphyromonas – pigmented anaerobes
 - black or brown color colonies
 - Colonies, pus & even dressings from wounds infected with this bacillus gives a characteristic red fluorescence when exposed to UV light
 - Infections above neck

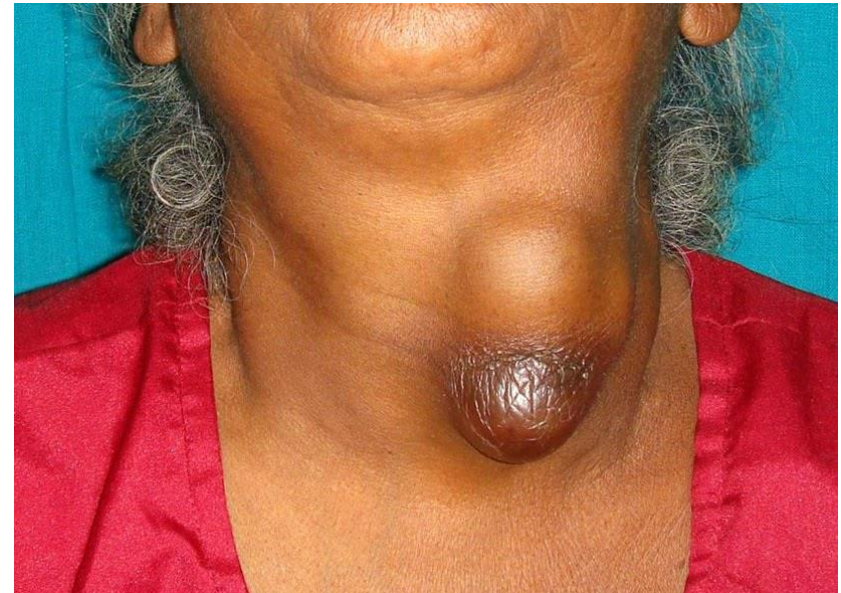
B. Fragilis

Prevotella & Porphyromonas
pigmented anaerobes

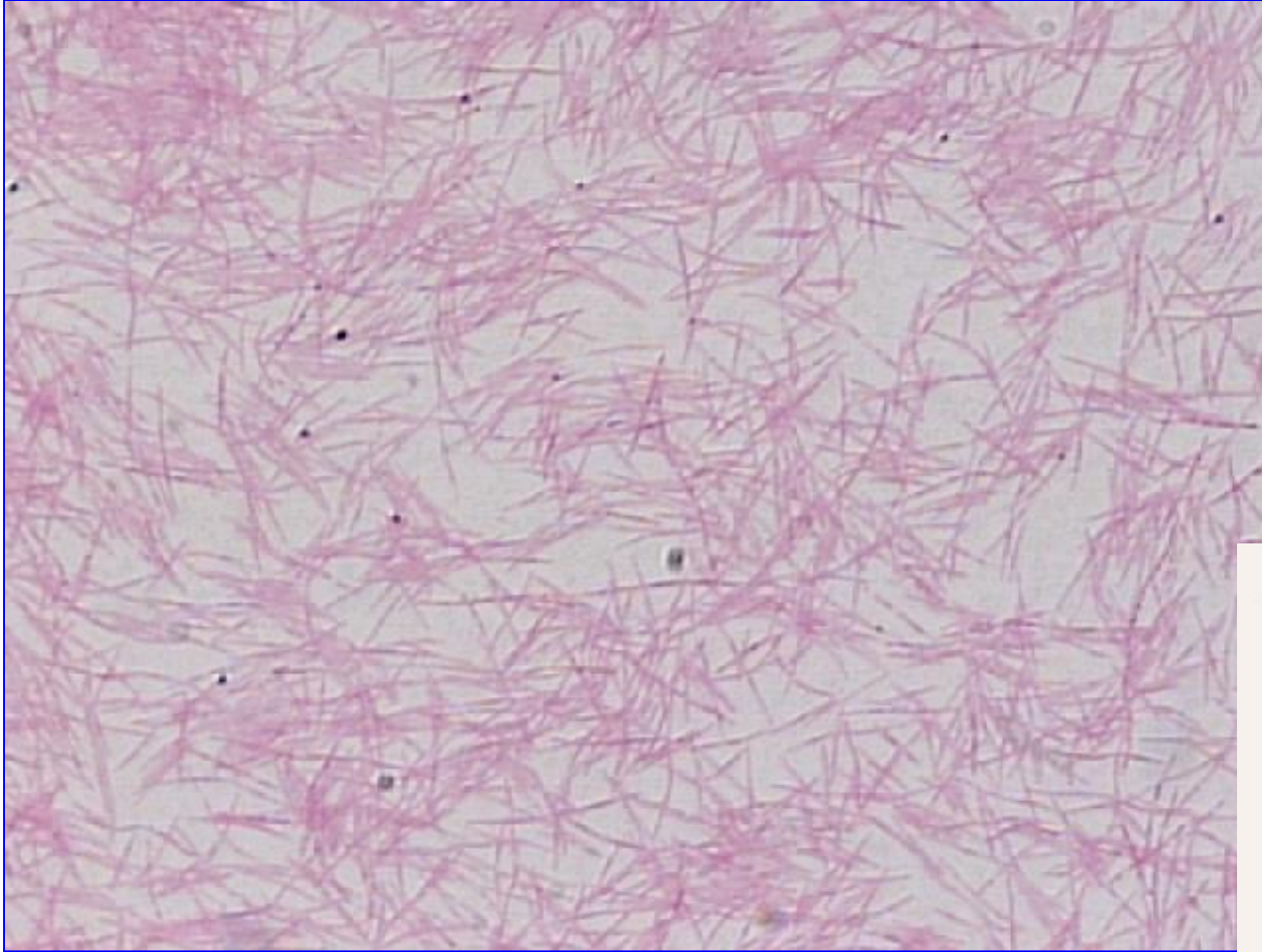


Gram-Negative Bacilli cont.....

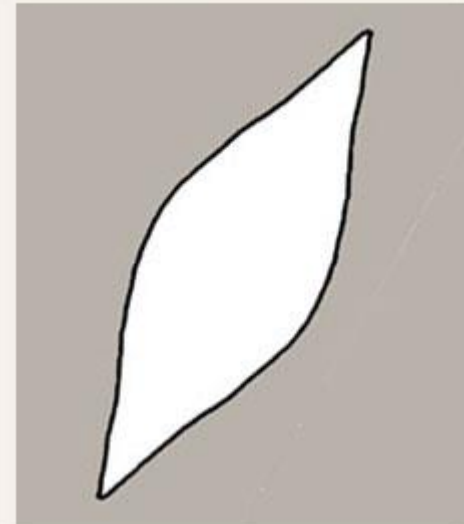
- *Fusobacterium* species
 - Oral origin (mainly)
 - Commensals of upper respiratory and gastrointestinal tracts.
 - *Fusobacterium necrophorum*
 - Causes Lemierre's syndrome /human necrobacillosis-thrombophlebitis of the internal jugular vein
- *Leptotrichia* species
 - *Leptotrichia buccalis*
 - “Vincent gingivitis”



Fusobacterium spp.



fusiform



Infections by Gram negative anaerobic bacilli

Bacteroides fragilis

Brain abscess, intra abdominal abscess, infections of female genitalia, cellulitis, diabetic ulcer, necrotizing fascitis
septicaemia

Prevotella melaninogenica

Lung or liver abscess, empyema, pelvic infections in females, breast abscess, wound infections

Porphyromonas

Dental root canal infections, periodontal disease

Fusobacterium necrophorum

Fusobacterium nucleatum

Lemierre's syndrome, Aspiration pneumonia, lung/ liver abscess, oral infections, chronic sinusitis, abdominal infections



Vincent angina- fusobacterium and *Borrelia vincentii*



Periodontitis



Anaerobic polymicrobial cellulitis



Necrotizing fascitis

Gram positive anaerobes

- *Propionibacterium acnes*
 - Skin commensal
 - Contaminant of blood culture
 - But can cause
 - Prosthetic infections, infections of shunts
 - Brain abscess, endocarditis
 - Conjunctivitis, keratitis
 - Acnes
- *Lactobacillus*
 - Commonest vaginal flora
 - Low pathogenicity
- *Mobiluncus*
 - Cause bacterial vaginosis (polymicrobial)



Infections by Actinomyces

- Oral cervicofacial disease
 - Pus containing granules discharge from sinuses
- Thoracic disease= TB
 - Empyema → break through chest wall → sinus dx
- Abdominal disease = tumor
 - Abscess commonly in RIF
- Peivic
 - Following IUCD
- Actinomycetoma
 - chronic subcutaneous infection
 - presence of abscesses, nodules, and sinuses that drain pus containing sulphur granules
 - *A. israelii*, *Nocardia brasiliensis*, *Actinomadura madurae*, and *Streptomyces*
- Brain, breast abscess

• **Due to disruption of mucosal barrier**

- Dental extractions
- Trauma to face/ chest
- Post surgery
- Foreign body- IUCD

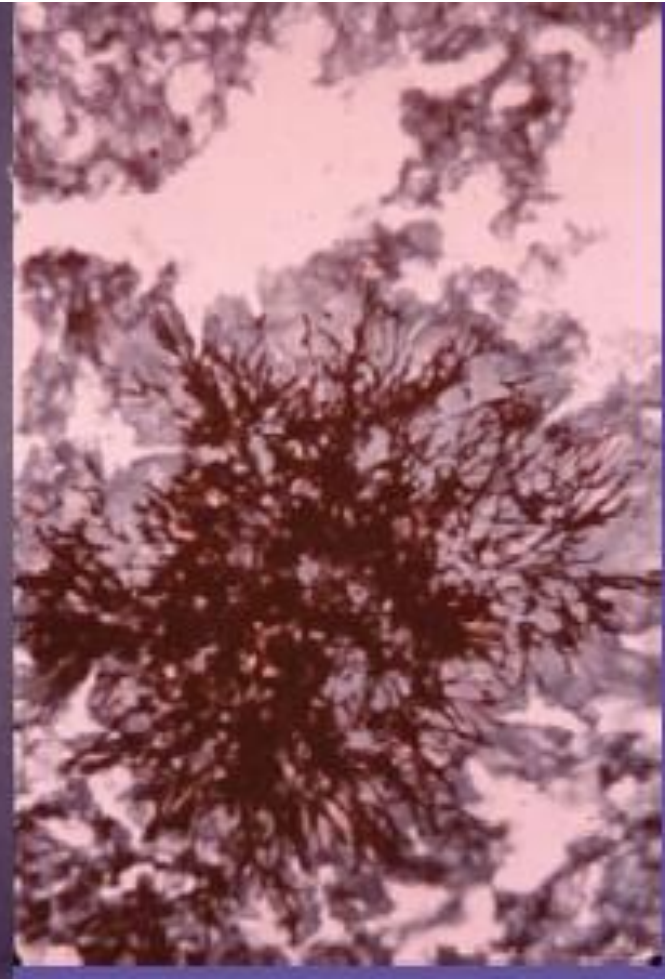
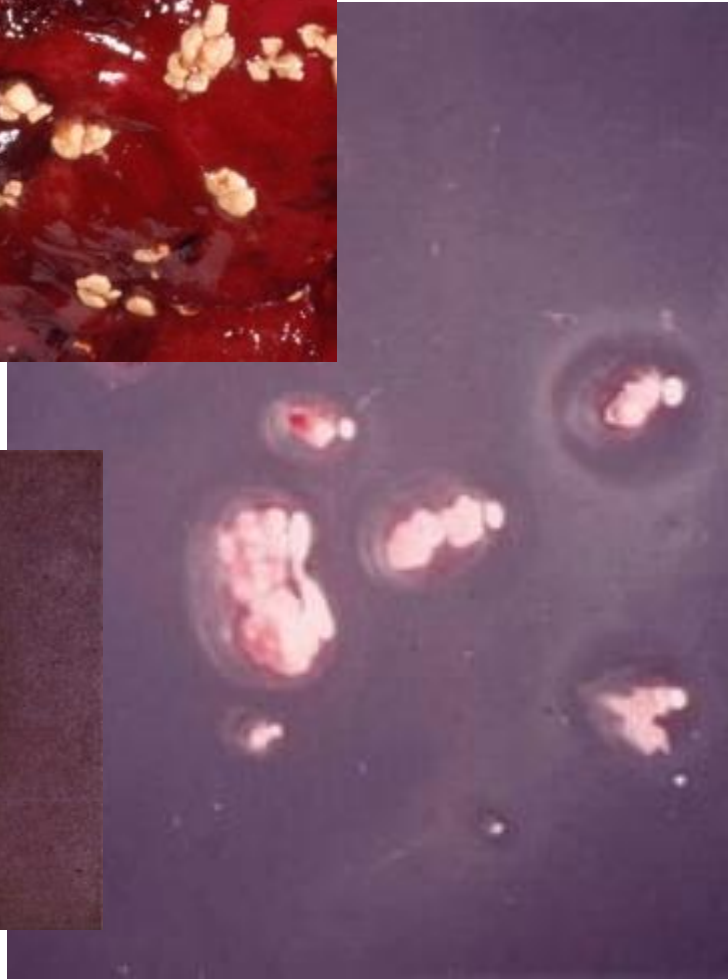
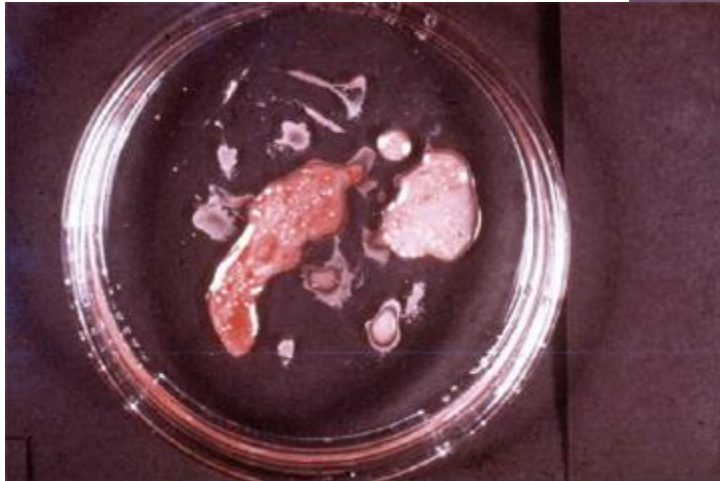
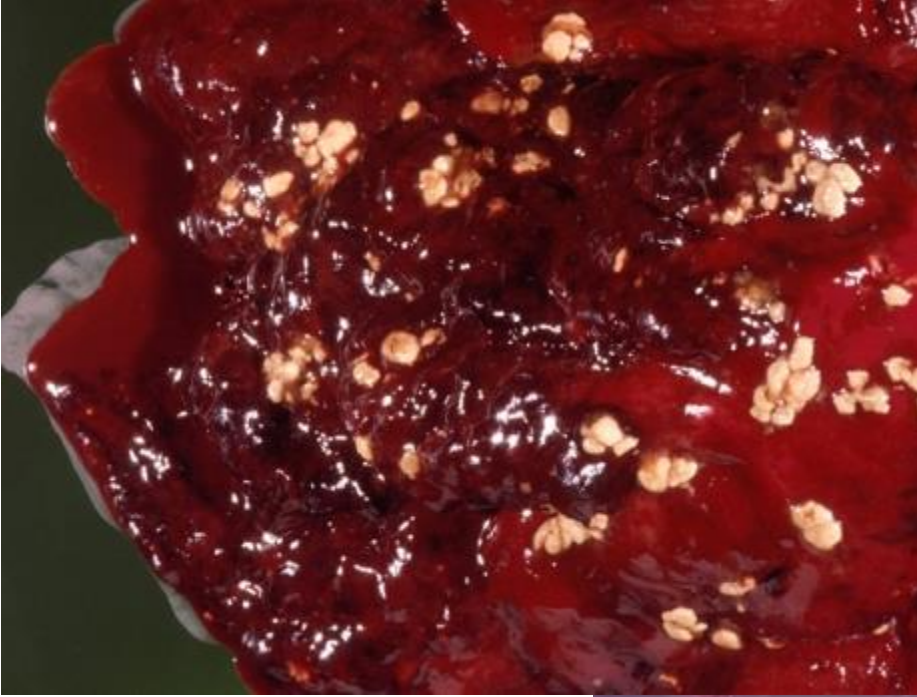


San Fran-
weed stem

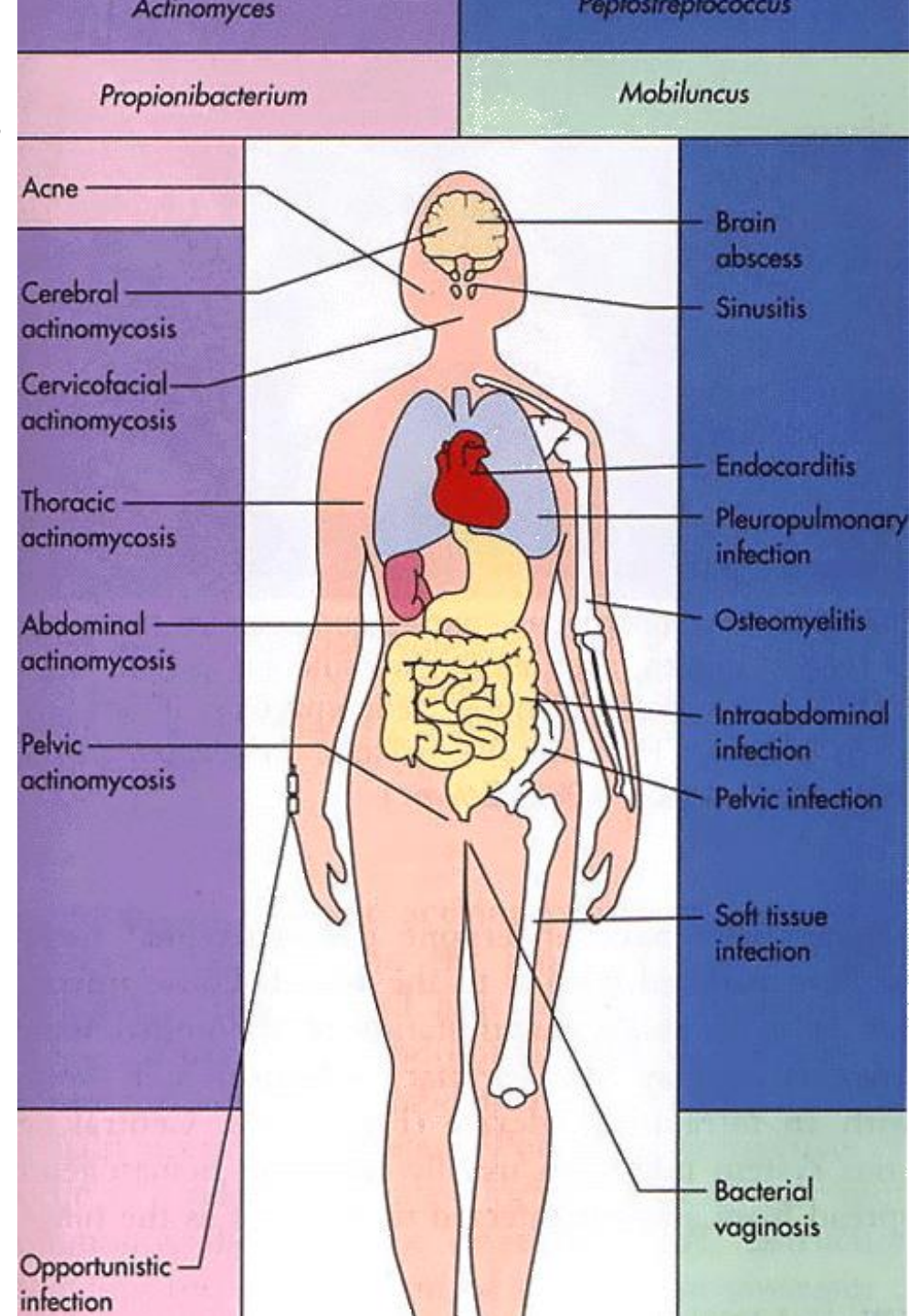
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Sulphur granules



Gram positive anaerobes

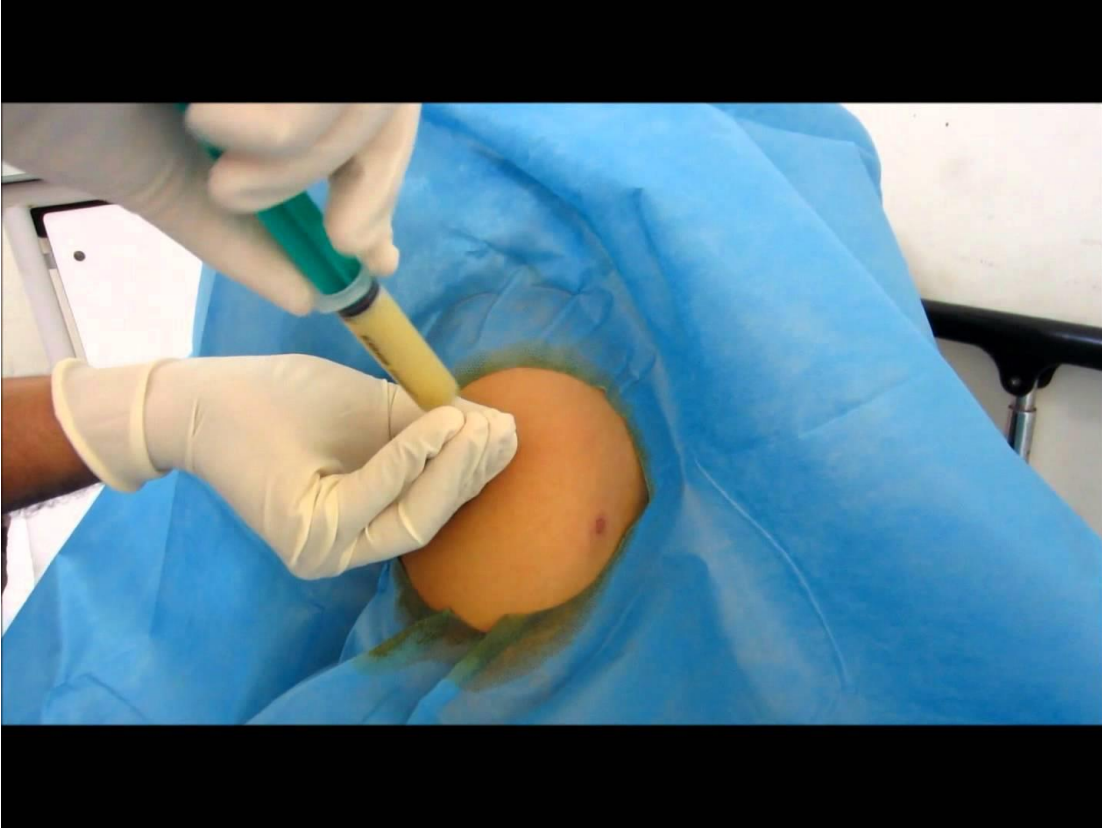


Diagnosis of Anaerobic infections

- Specimens - Deep aspirates, tissues, biopsies
 - Any specimen collected on a swab is usually not acceptable because of the possibility of having normal anaerobic organisms
- Collection & transport
 - Pre-reduced transport medium
 - Gassed out vials
 - Needle & syringe with rubber bung
 - Must be transported with minimum exposure to oxygen

Diagnosis of Anaerobic infections

- Aspirates
 - Should be collected with needle and syringe
 - Excess air expressed from syringe
- Tissue
 - must be placed in an oxygen-free transport bag or vial
- Blood / BM
 - anaerobic blood culture bottles
- CSF- anaerobic container
- BAL/ Bronchial Wash/PCTA



Diagnosis of Anaerobic infections

- Methods
 - Macroscopic examination of pus
 - Colour (black pus for pigmented pathogens)
 - Smell
 - Presence of granules
 - Microscopy- Gram stain
 - Presumptive ID- ex. Actinomyces, fusobacterium
 - Help in gas gangrene- brick shape bacilli with no pus cells

Diagnosis of Anaerobic infections

- Methods

- Culture

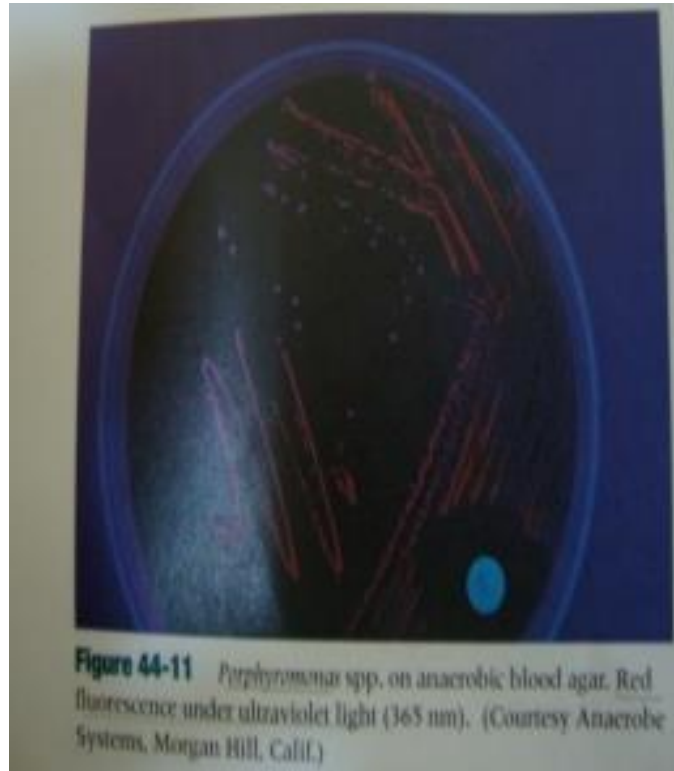
- Non selective agar- anaerobic blood agar
 - Selective agar – Bacteroides Bile Esculin agar (BBE)
 - Anaerobic broths- Cooked meat broth, thioglycollate broth
 - Incubate anaerobically- anaerobic jars, anaerobic chambers
 - Slow growth- incubate >48 hrs
 - ID- Colony morphology, Gram stain, Biochemical tests
 - UV light (Wood's Lamp) over colonies
 - Brick red fluorescence (Prevotella), yellow (fusobacterium)

- Gas Liquid Chromatography (GLC)

- Detection of fatty acid end products (butyric acid, acetic acid, lactic acid)
 - Can be done directly on pus and other specimens or from culture



Culture on BBE



Fluorescence under
Wood's lamp (UV)



GLC



Anaerobic GasPak jars



Anaerobic chamber

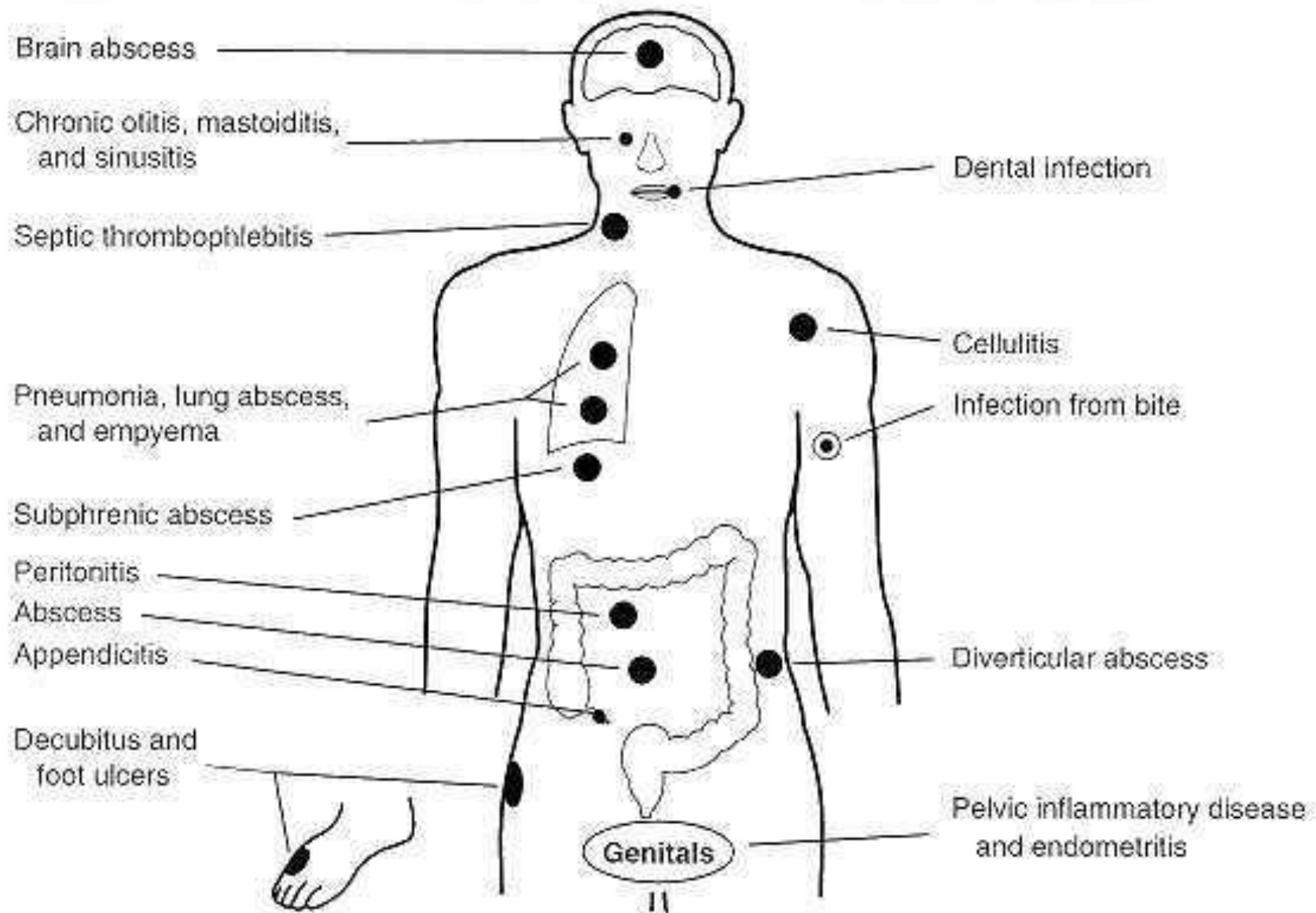
Antimicrobial Susceptibility

- Not routinely performed
 - Drugs of choice
 - Metronidazole- cover all anaerobes
 - Penicillin
 - DOC for Actinomyces- given for a long period
 - not effective for Bacteroides (beta lactamase)→ Co-amoxiclav is effective)
 - Co-amoxiclav
 - Clindamycin
 - Chloramphenicol
 - Cephalosporins

All anaerobes are resistant to aminoglycosides

Management of anaerobic infections

- Surgical drainage of abscess, excision of necrotic tissues
- ABx treatment
 - For mixed infections cover for both aerobes and anaerobes
- Hyperbaric oxygen
 - Oxygen is forced into necrotic tissues, killing anaerobes



Clostridium

- Gram positive
- Rods
- Endospore formers
 - asporogenous
- Obligate Anaerobes
 - Aerotolerant spp *C. perfringens*, *C. tetani*, *C. botulinum*, *C. difficile*
- Soil organisms

Tetanus

- *C. tetani*
- *Gram pos bacilli*
- *Spore forming- exogenous*
- Due to powerful neurotoxin (tetanospasmin)
- Clinical presentation
 - Trismus
 - Rhisus sardonicus
 - Opisthotonus
 - Violent spasms
 - Convulsions
 - Local tetanus
 - Neonatal tetanus
 - Otogenic tetanus
 - Septic abortions

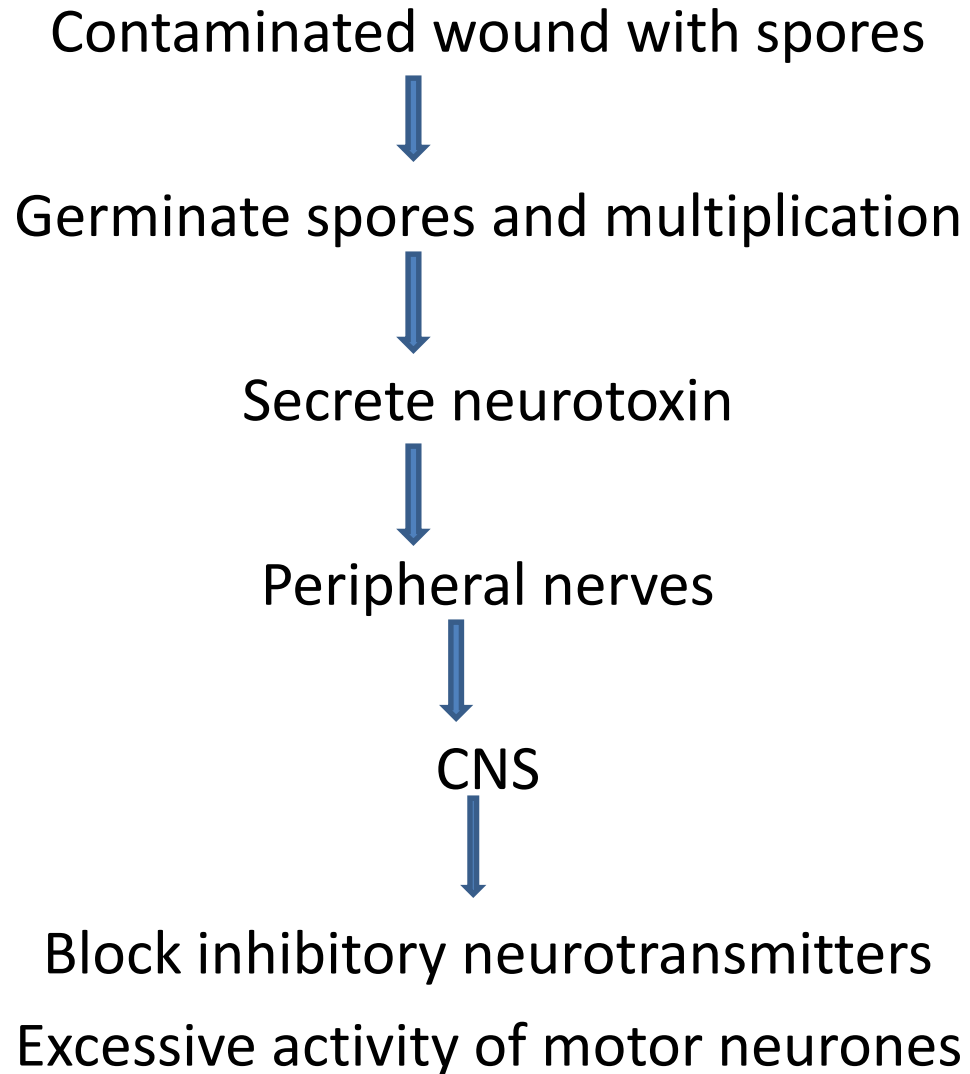


Clostridium tetani Gram Stain

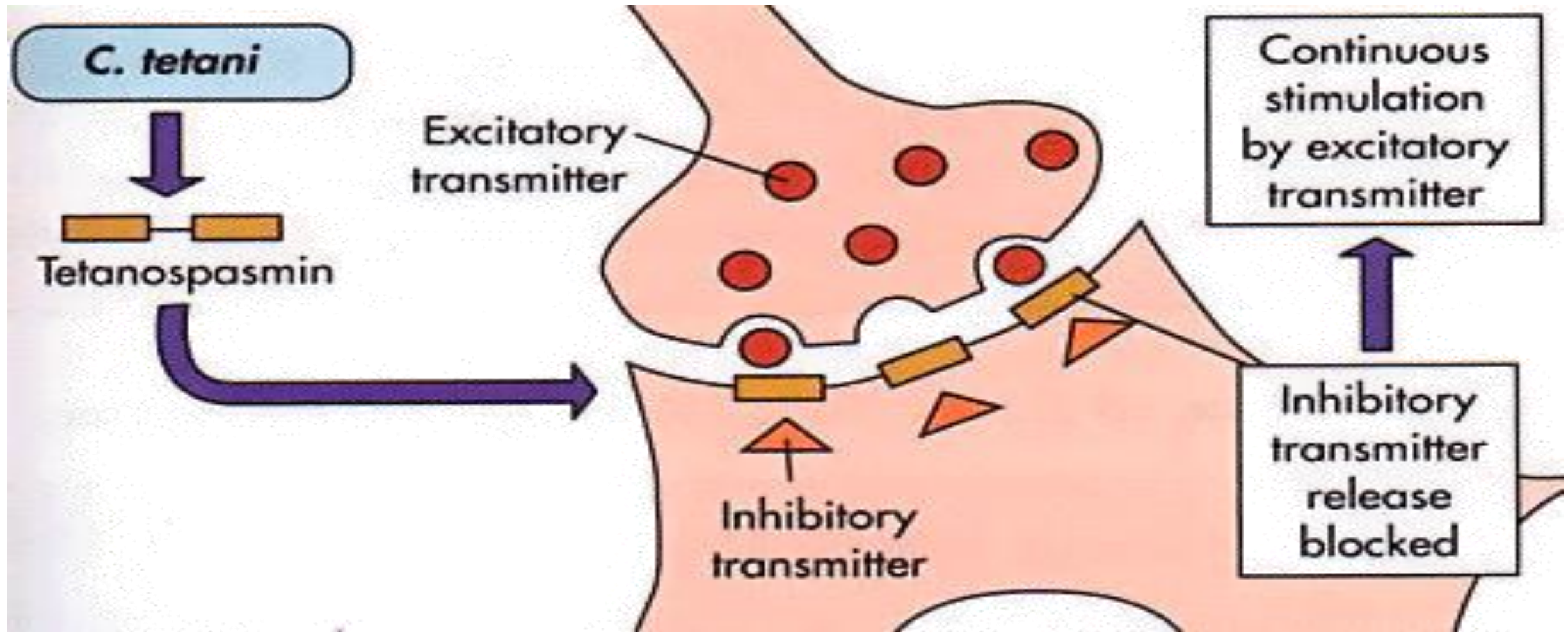


NOTE: Round terminal spores give cells a “drumstick” or “tennis racket” appearance.

Tetanus- pathogenesis



Mechanism of Action of Tetanus Toxin

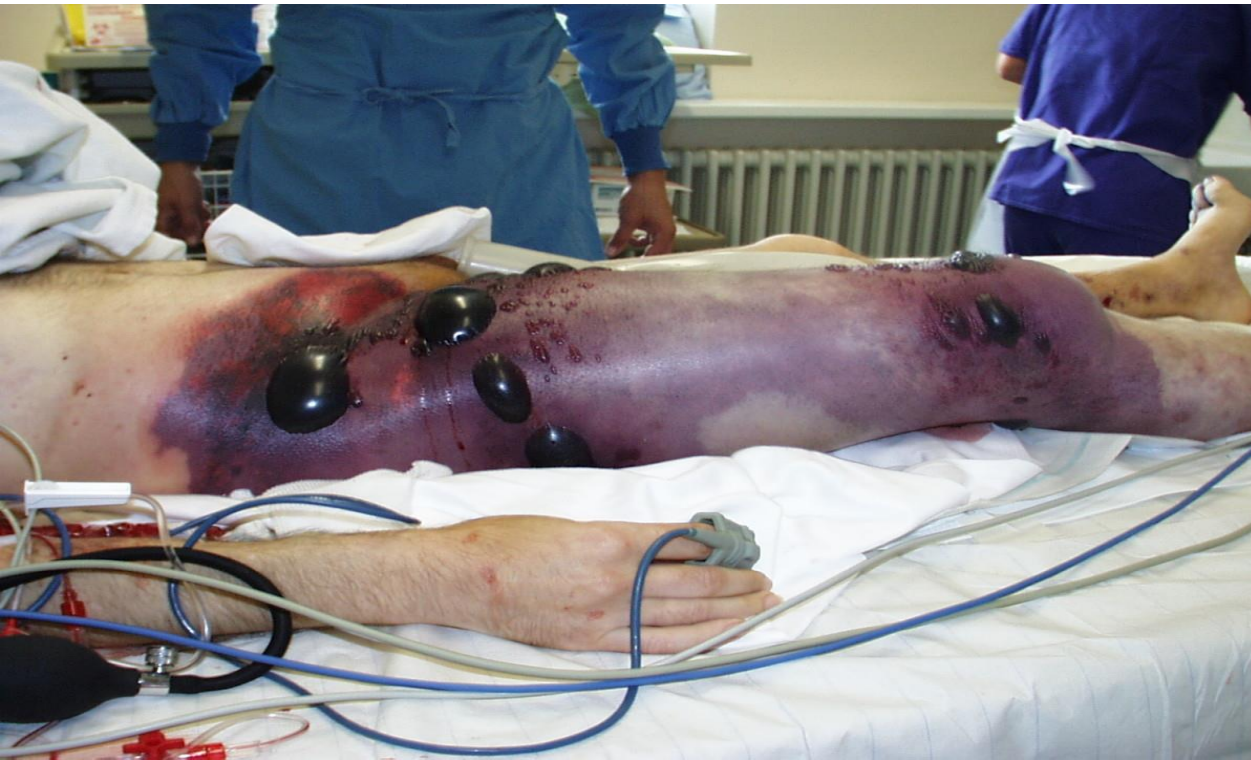


Diagnosis, treatment and prevention

- Specimens- wound exudate
- Culture- anaerobically (Blood agar, Cooked meat broth)
- Treatment
 - Tetanus hyper-immunoglobulin (anti-toxin)
 - Penicillin
 - Wound debridement
 - Tetanus toxoid
- Prevention
 - Pre-exposure – TT, DPT, DT
 - Post- exposure- TT, penicillin prophylaxis

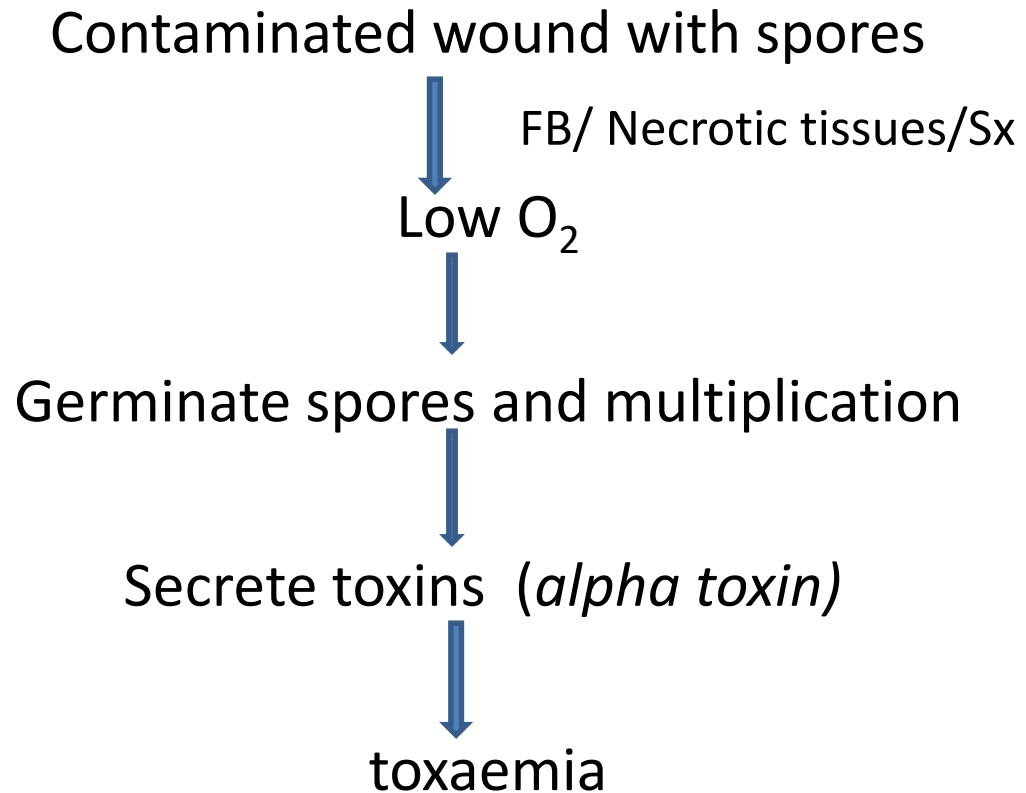
Gas gangrene

- *C. perfringens*- GPB, spore
- Major toxin- alpha toxin
(phospholipase/ lecithinase)



- Rapidly spreading oedema of tissues
 - Myositis
 - Necrosis/ crepitus
 - Gas production
 - Toxaemia/ shock
-
- Complication of surgery
 - Septic abortions, gangrenous appendicitis

Gas gangrene- Pathogenesis



Stage 2

Muscle necrosis and further exotoxin production and organism spread (*C. perfringens* and other species)

Edema

Gas pockets

Stage 3

Gas
gangrene

Blood vessel compression

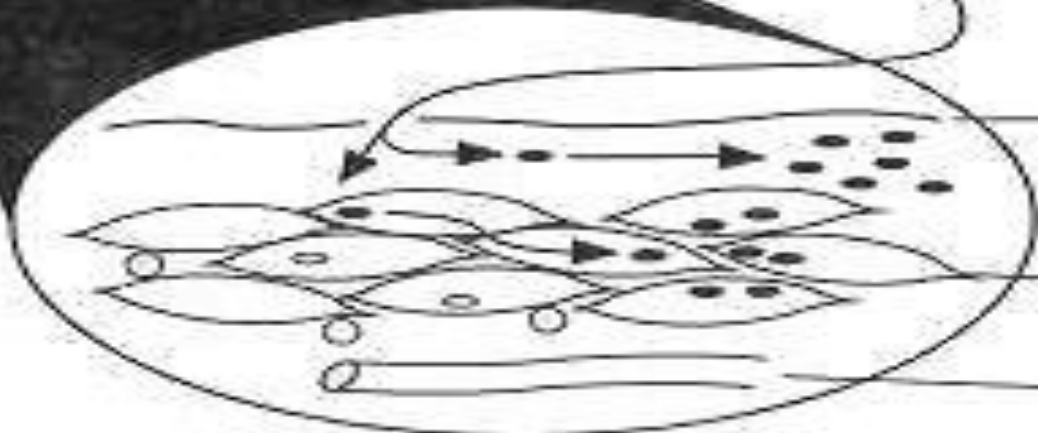
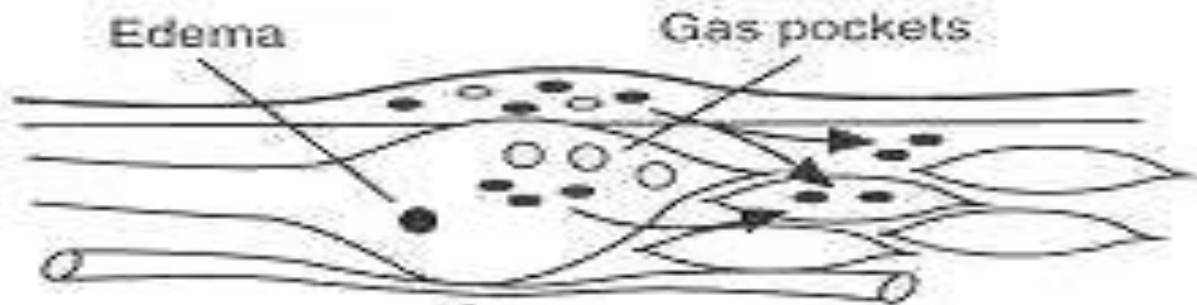
Stage 1

Organisms accompany
foreign object penetrating
skin or muscle

Dermis

Muscle

Blood vessel



Clostridial Cellulitis



C. Perfringens - Food poisoning

- incubation 8-24hrs.
- Abdominal cramps, watery diarrhea
- lasts less than 24hrs.
- Contaminated meat (left overs)

Micro & Macroscopic C. perfringens

NOTE: Large rectangular gram-positive bacilli



NOTE: Double zone of hemolysis

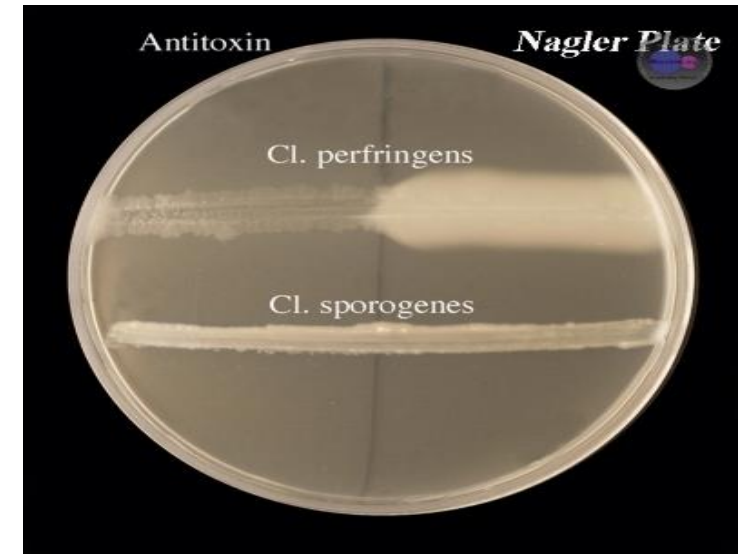


Inner beta-hemolysis = θ toxin

Outer alpha-hemolysis = α toxin

Diagnosis and treatment

- Specimens- wound tissues
- Direct smear (GS)- GPB, lack of inflammatory cells
- Culture- anaerobically (BA, CMB)
- Toxin detection- Nagler reaction
- Treatment
 - Surgical debridement
 - High dose penicillin + metronidazole + gentamycin
 - Novel therapies – Hyperbarbic oxygen
IV immunoglobulins



C. perfringens Nagler Reaction



NOTE: Lecithinase (α -toxin; phospholipase) hydrolyzes phospholipids in egg-yolk agar around streak on right. Antibody against α -toxin inhibits activity around left streak.

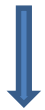
Botulism

- *C. botulinum*

Contaminated food (canned food, honey)



*Germination of spores &
Toxin production*



Ingestion of toxin

SI → Blood →

Prevent release of Acetylcholine →

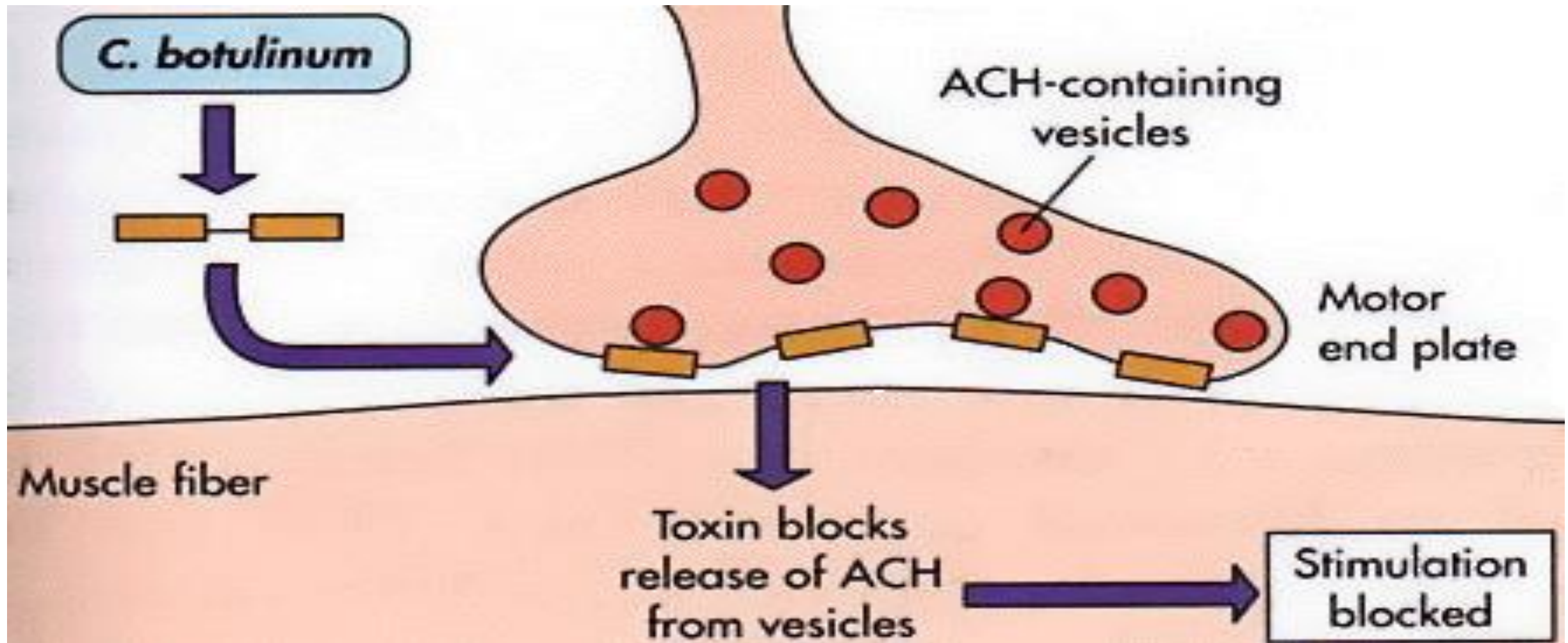
Flaccid paralysis

Highly toxic
7 types –A to G
A, B, E commonest
Clinical use as an
anticholinergic



Nerve paralysis
Blurred vision
Cardiac failure
Respiratory failure

Mechanism of Action of Botulinum Toxin



Clostridium difficile

- Gram positive, Spore forming
- Ubiquitous
- Colonize GI tract of 5% of healthy people
- Spores detected in hospital environments with infected pts
- Mainly exogenous acquisition of spores
- Overgrowth occurs following exposure to antibiotics
- Produce toxins
 - Enterotoxin (toxin A)
 - Cytotoxin (toxin B)
 - Hyalurodinase

Antibiotic associated diarrhoea/ colitis

- *C. difficile*

Use of antibiotics (ampicillin, clindamycin, cephalosporines- commonly, but any antibiotic can cause)



Inhibit normal flora



Multiplication of C. dif

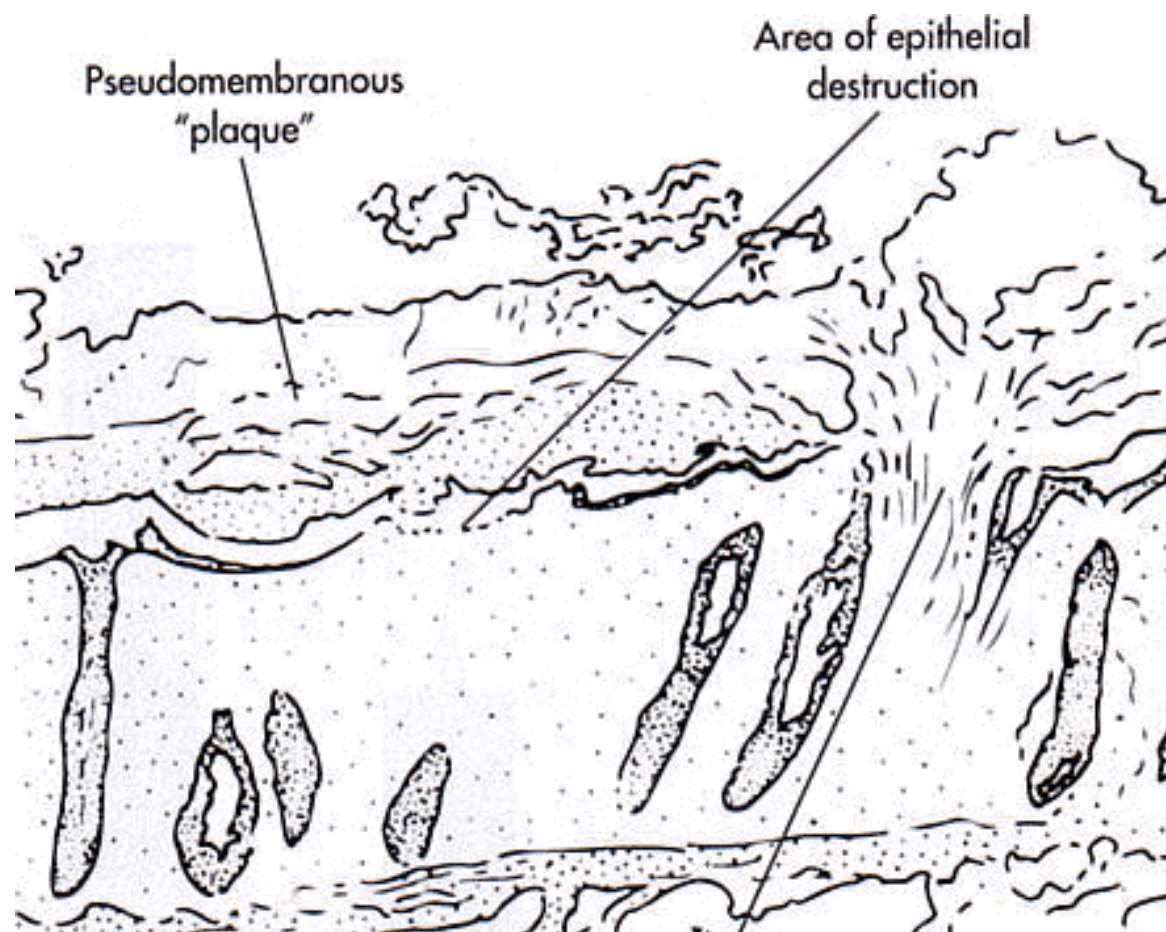


Production of Toxin



Inflammation of colonic mucosa

Pseudo-membrane formation, diarrhoea, colitis



Diagnosis and treatment

- **Tests**

- Toxin testing (cytotoxin)
- Stool culture
- *C.difficile* antigen -latex agglutination

- **Treatment**

- Discontinue culprit antibiotics if possible
- Fluid and electrolyte replacement
- Specific treatment
 - Oral metronidazole – in mild/moderate infection
 - Oral vancomycin – in severe infection
 - Faecal transplant - SDL
 - Relapses are common due to resistant spores

Summary

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 - Habitat
- Anaerobic Infections
- Specimens for diagnosis
- Transport
- Diagnostic methods
- Management