# Lecture Title:

## Anaerobes of clinical Importance

(Foundation Block, Microbiology)

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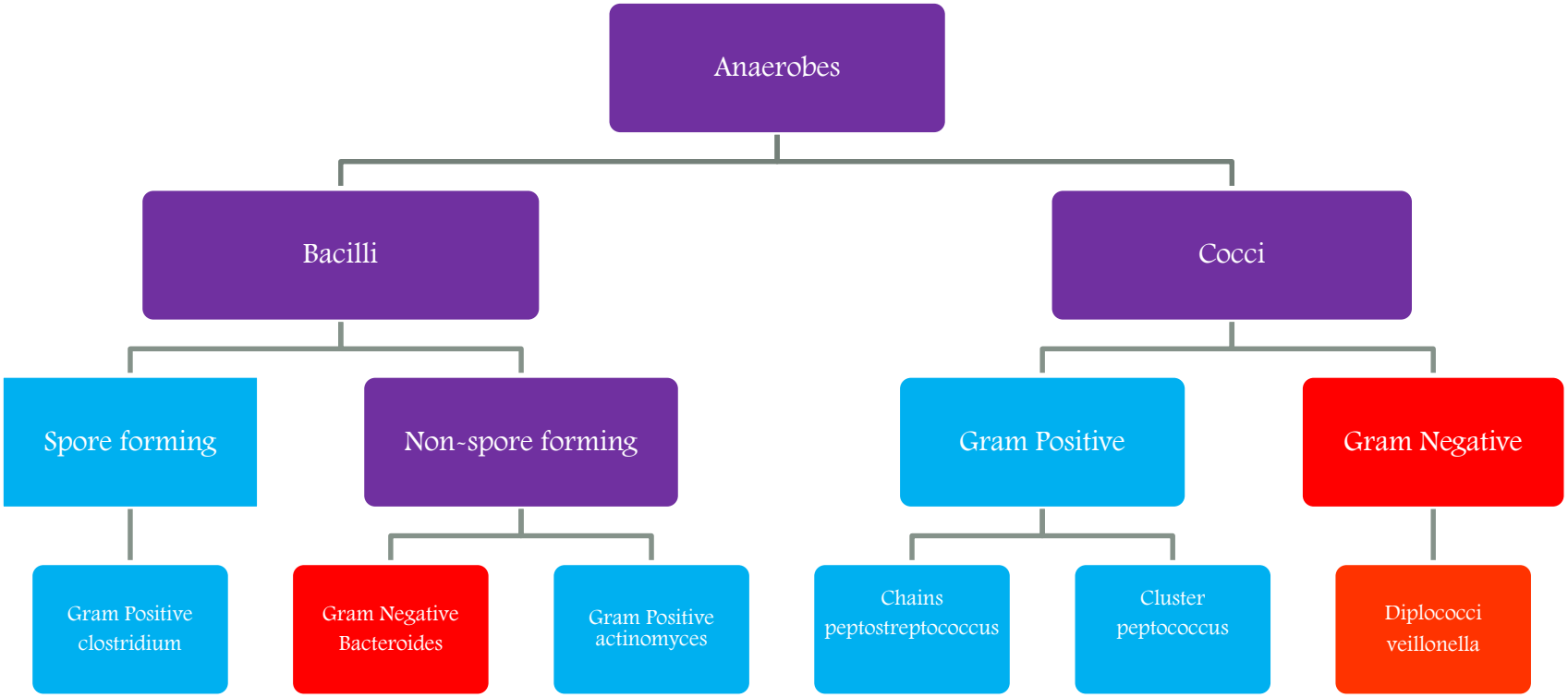
# LECTURE OBJECTIVES

- **By the end of this lecture the student should be able to:**
- Describe anaerobic bacteria including their sensitivity to oxygen and where they may be found in the environment and the human body.
- Differentiate the various types of anaerobes with regard to atmospheric requirement (i.e. obligate anaerobes, Facultative anaerobes and aerotolerent anaerobes.
- Describe how anaerobes, as part of endogenous microbiota, initiate and establish infection.
- Name the endogenous anaerobes commonly involved in human infection.

# LECTURE OBJECTIVES

- Recognize specimens that are acceptable and unacceptable for anaerobic culture.
- Give the clues(sign and manifestations) to anaerobic infection, name the most probable etiologic agents of the following(Wound botulism, gas gangrene,tetanus,Actinomycosis,Pseudomembranous colitis and bacterial vaginosis)
- Describe the microscopic and colony morphology and the results of differentiating anaerobic isolates.
- Discuss antimicrobial susceptibility testing of anaerobes including methods and antimicrobial agents to be tested.
- Describe the major approaches to treat anaerobic-associated diseases either medical or surgical.

# CLASSIFICATION



# ANAEROBIOSIS

- Contain flavoprotein so in the presence of oxygen produce H2O2 which is toxic
- Lack cytochrome -cannot use oxygen as hydrogen acceptor
- Most Lack Catalase & Peroxidase
- Some lack enzyme superoxide dismutase so many killed
- Peroxide and toxic radicles enzyme like fumarate reductase must be in reduced form to work

# HABITAT :

These organism are normal flora in:

A. Oropharynx *eg.*

1. *Fusobacteria*
2. *Provetella melaninogenicus*
2. *Veillonella*

B. Gastrointestinal tract

- Found mainly in the large colon in large numbers
- Total number of anaerobes = $10^{11}$
- While all aerobes (including E. *coli)* = $10^{14}$
- examples are

1. *Bacteroides fragilis*
2. *Bifidobacterium species*

C. Female genital tract (mainly in the vagina)

# FEATURES OF ANAEROBIC INFECTIONS

**Infections are always near to the site of the body which are habitat.**

1. Infection from animal bites.
2. Deep abscesses
3. The infections are also polymicrobial
4. Gas formation, foul smell
5. Detection of "Sulphur granules"' due to actinomycosis
6. Failure to grow organism from pus if not culture anaerobically.
7. Failure to respond to usual antibiotics.

# HOW DOES THE INFECTION BEGIN ?

- Disruption in barrier
  - Trauma
  - Surgery
  - Cancer
- Disruption in blood supply
  - Drop in O2 content of tissue.
  - Decrease in Eh potential
  - Tissue necrosis

# WHAT ARE THE INFECTION CAUSED BY THESE ANAEROBIC ORGANISMS I

- Post operative wound infection
- Brain, dental, lung abscess
- Intra abdominal abscess, appendicitis, diverculitis
- Infection of the female genital tract: Septic abortion, puerperal infection and endometritis , pelvic abscess or breast abscess
- Diabetic foot infections and pilonidal sinus

# LABORATORY DIAGNOSIS:

When anaerobic infection is suspected;

a) Specimens have to be collected from the site containing necrotic tissue.
b) Pus is better than swabs.
c) Specimens has to be send to the laboratory within 1/2 hour why?
d) Fluid media like cooked meat broth are the best culture media.
e) Specimens have to incubated anaerobically for 48 hours.

# TREATMENT:

- *Bacteroides fragilis* is always resistant to penicillin.
- But penicillin can he used for other anaerobes
- Flagyl (metronidazole) is the drug of choice.
- Clindamycin can also be used.

# ANAEROBIC NON SPORE FORMING GRAM POSITIVE BACILLI

| ORGANISM | DISEASE |
|---|---|
| *ACTINOMYCES SPP* | ACTINOMYCOSIS |
| *PROPIONIBACTERIUM SPP* | ACNE |
| *MOBILUNCUS SPP* | BACTERIAL VAGINOSIS |
| *LACTOBACILLUS SPP* | ENDOCARDITIS |
| *EUBACTERIUM SPP* | - |
| *BIFIDOBACTERIUM SPP* | - |

# ACTINOMYCOSIS

- Actinomyces are branching  anaerobic or microaerophilic Gram positive bacilli
- Source of the infection is normal flora and the host usually normal host
- Primary site of the infection is mouth, lung, appendix, uterus with IUD (chronic infection)
- Infection can spread to the brain, liver, bone and blood
- Diagnosis by Gram stain with sulfur granules and growth of molar tooth colonies
- Treatment penicillin, clindamycin or tetracycline

# ANAEROBIC GRAM NEGATIVE BACILLI

- **BACTEROIDES**
- **FUSOBACTERIUM**
- BUTYRIVIBRIO
- SUCCINOMONAS

- STRICT ANAEROBE PLEOMORPHIC (COCCO BACILLI)GRAM NEGATIVE BACILLI

| BACTEROIDES FRAGILLIS GROUP | BACTEROIDES SPECIES OTHER THAN B. FRAGILIS GROUP |
|---|---|
| ACCOUNT FOR 1/3 OF ALL ISOLATES<br>GIT TRAC | LESS COMMON<br>ORAL CAVITY |
| *B. FRAGILIS-B. VULGARIS-B.THETAIOTAMICRON*<br>*B. UNIFORMIS* | *PREVOTELLA*<br>*PORPHYROMONAS* |
| RESISTANT TO 20% BILE | BILE SENSITIVE |
| RESISTANT TO MANY ANTIBIOTICS<br>PENICILLIN, KANAMYCIN, VANCOMYCIN,<br>COLISTIN – AND MANY MORE | RESISTANT TO KANAMYCIN ONLY |
| NO PIGMENTATION OF COLONIES OR FLUORESCENCE | SOME PIGMENTED |

| OTHER ANAEROBIC GRAM NEGATIVE BACILLI | ANAEROBIC GRAM POSITIVE COCCI | | ANAEROBIC GRAM NEGATIVE COCCI |
|---|---|---|---|
| BACILLI | CHAINS | CLUSTERS | SHEET-LIKE-or Pairs |
| • **FUSOBACTERIUM NECROPHORUM** | PEPTOSTREPTOCOCCUS | PEPTOCOCCUS | VEILLONELLA PARVULA |
| PERITONISILLAR →INTRNAL JUGULAR VEIN THROMBOSIS→EMBOLI TO THE LUNG LEMIERRE SYNDROM | BRAIN ABSCESS | - | - |

# LARGE GRAM POSITIVE SPORE FORMATION BACILLI : CLOSTRIDIUM SPECIES

| CLOSTRIDIUM SPECIES | MAJOR DISEASE |
|---|---|
| *Cl. perfringens* and other e.g septicum | Gas gangrene |
| *Cl. tetani* | Tetanus |
| *Cl. botulinum* | Botulism |
| *Cl. difficile* | 4. Toxic enterocolitis (Pseudomembernous colitis) |

# *Clostridium perfringens (C. welchii)*

- large Gram positive bacilli with bulging sub terminal endospores
- **Clinical Features**
- Can leads to the following diseases
- 1) Wound infection after wound Contamination can lead to Gas Gangrene - most serious disease
- 2) Gas Gangrene of the uterus after criminal abortion
- 3) Food Poisoning : Spores are swallowed then germinate in gut after 18 hours(Toxin production) lead to abdominal pain and diarrhoea

# *Clostridium perfringens (Cl . welchii)*

- **Pathogenesis:**
- Traumatic open wounds or compound fractures lead to muscle damages and contamination with dirt etc,
- Mainly in war wounds, old age, low blood supply and amputation of thigh (required prophylaxis with penicillin

# *Clostridium perfringens (C . welchii)*

- **Laboratory diagnosis**
- Smear Gram stain Large Gram positive bacilli with few or no WBCs
- Culture
- Blood agar with haemolytic colonies (double zone of haemolysis )
- Cooked meat medium
- Gives the NAGLAR'S Reaction & toxin neutralization on Egg yolk medium & toxin is a phospholipase

- **Treatment and Prevention**
- Remove dead tissue , debris and foreign bodies .Penicillin and hyperbaric oxygen in some cases

# *Clostridium .tetani* (Tetanus)

- Anaerobic Gram positive bacilli with terminal spore( called drum Stick appearance)
- Lives in soil and animal feaces. e,g horse and any wound can infected if contaminated by spores

# *Clostridium .tetani* (Tetanus)

- **Clinical Features**
- Incubation period 1-3 weeks (time from infection to the appearance of symptoms)
- Face & neck wounds are more dangerous
- Symptoms: local (not common), cephalic (rare), generalized (most common)
- Painful muscle spasm around infected wound and Contraction of muscles in the face called **Trismus** (Lockjaw) , **Risus Sardonicus (facial muscle)**
- **Opisthotonus (Araching of Back)** in children.
- O***pistho*** meaning "behind" and ***tonos*** meaning "tension", due to extrapyramidal effect and is caused by spasm of the axial along the spinal column .

# *Clostridium .tetani* (Tetanus)

## Pathogenesis

- Mainly due to **tetanospasmin** which is powerful exotoxin (protein) .
- This organism does not lead to invasion or Bacteraemia . Its function to inhibits transmission of normal inhibitory messages from central nervous system at anterior horn cells of cord.

## Laboratory Diagnosis

- Mainly by clinical and it is strict anaerobe very motile , spread on agar.

# *Clostridium .tetani* (Tetanus)

**Treatment**

- Cleaning of wound and removal of Foreign body
- Specific by antitoxin form horse serum but it can lead to anaphylaxis & shock must be tested first or human immunoglobulin.
- Antibiotics : Penicillin.
- Supportive treatment by keeping the patient in dark pace, fluids and sedative valium

**Prevention**

- by vaccination

# *Clostridium botulinum*

- Found in soil ponds and lakes
- Toxin is exotoxin (protein) heat labile at 100 °C and resist gastrointestinal enzymes
- It is the most powerful toxin known Lethal dose 1 µg human and 3 kg kill all population of the world .It dictated for by lysogenic phage

**Botulism**

- From canned food., sea food e_g. salmon when it is not well cooked (Spores resist heat at 100 °C ) →then multiply and produce toxin

# *Clostridium botulinum*

## Clinical features

- Abnormal eye movement as if cranial nerve affected when bulbar area of the brain affected.
- Finally the patient might develop respiratory and circulatory collapse
- Infantile Botulism
  - Ingestion of ***Spores*** → *germination in the* ***gut*** → ***Botulism .Child present with w***eek child, cranial nerve and constipation

## Pathogenesis

- Attacks neuromuscular junctions and prevents release of acetylcholine that can leads to paralysis

# *Clostridium botulinum*

## Laboratory diagnosis

- Suspected food from the patient faeces culture or serum toxin detection by mice inoculation after weeks → paralysis and death

## Treatment

- Mainly supportive and horse antitoxin in sever cases

## Prevention

- Adequate pressure cooking autoclaving and heating of food for 10 minutes at 100 $^{O}C$

# Clostridium Difficile

Normal flora in gastroentestinal tract

## Clinical features

**PSEUDOMEMBRANE COLITIS** is the clinical manifestation of this disease which composed of bacteria , fibrin , WBCs and dead tissue cells

Sever dehydration , intestinal obstruction and perforation are some of complication of this syndrome

- **Pathogenesis**
- after exposure to antibiotics and killing of other normal flora, this organism will multiply witch then produce toxin that has two components
- A–Subunit enterotoxin (cause diarrhea)
- B-Subunit Cytotoxic ( kill the cells ie necrosis)

# *Clostridium Difficile*

**Laboratory diagnosis**:

This organism hard to grow in the laboratory required special media and growth of the organism in solid media required cell line culture to illustrate cytotoxicity of the organism. The simplest method for diagnosis by detection of the toxin in the stool by immunological testing (ELISA) or molecular testing PCR

# *Clostridium Difficile*

**Treatment** :

Metronidazole or and oral vancomycin in sever cases

**Prevention**:

This organism form spores and hard to control in the hospital because they are resistant to alcohol decontamination ( use Na hypochloride instead).

- Patient need to be isolated and contact need to be screened to find out if they carrying the toxic strain of the bacteria.

# Reference book and the relevant page numbers..

**Sherries Medical Microbiology, an introduction to Infectious Diseases.** Latest edition, Kenneth Ryan and George Ray. Publisher: Mc Graw Hill.

# THANK YOU

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