

Lecture Title:

Anaerobes of clinical Importance

(Foundation Block, Microbiology)

Lecturer name: *Dr. Ali Somily &*

Dr. Fawzia Al-otaibi

Department of Pathology, Microbiology Unit

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, Faculative 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

- Anaerobic spore forming bacilli (Clostridia)
- Gram negative bacilli non-sporing forming (Bacteroides)
- Anaerobic streptococci (*Peptostreptococcus*)
- Anaerobic staphylococcus (Peptococcus)
- Gram negative diplococci (Veillonella)
- Gram positive bacilli (Actinomyces)

ANAEROBIOSIS

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

HABITAT I:

These organism are normal flora in:

A. Oropharynx *eg. 1. Prevotella melaninogenicus 2.*

Fusobacteria 3. 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 OF BARRIERS
 - TRAUMA
 - OPERATIONS
 - CANCEROUS INVASION OF TISSUES
- DISRUPTION OF BLOOD SUPPLY
 - DROPS OXYGEN 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, diverticulitis
- 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 be used for other anaerobes
- Flagyl (metronidazole) is the drug of choice.
- Clindamycin can also be used.

ANAEROBIC NON SPORE FORMING BACILLI

- *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

ORGANISM GROUPS

GRAM NEGATIVE RODS

- BACTEROIDES
- PREVOTELLA
- PORPHYROMONAS
- FUSOBACTERIUM
- BUTYRIVIBRIO
- SUCCINOMONAS

BACTEROIDES

- STRICT ANAEROBE
- PLEOMORPHIC
- GRAM NEGATIVE BACILLI (COCCO BACILLI)
- NORMAL FLORA IN
 - OROPHARYNX
 - GASTROINTESTINAL TRACT
 - VAGINA

BACTEROIDES

GROUP = *B. FRAGILIS*, *B. VULGARIS*, *B. THETA IOTAMICRON*, *B. UNIFORMIS*

- ACCOUNT FOR 1/3 OF ALL ISOLATES
- RESISTANT TO 20% BILE
- RESISTANT TO MANY ANTIBIOTICS
 - PENICILLIN, KANAMYCIN, VANCOMYCIN, COLISTIN – AND MANY MORE
- NO PIGMENTATION OF COLONIES OR FLUORESCENCE

BACTEROIDES OTHER SP

BACTEROIDES SPECIES OTHER THAN B. FRAGILIS GROUP

- BILE SENSITIVE
- RESISTANT TO KANAMYCIN ONLY
- SOME PIGMENTED

OTHER GRAM NEGATIVE RODS

- FUSOBACTERIUM NECROPHORUM
- GRAM NEGATIVE BACILLI
- PERITONISILLAR → INTRNAL JUGULAR VEIN THROMBOSIS → EMBOLI TO THE LUNG
- PEPTOCOCCUS
- GRAM POSITIVE COCCI IN CLUSTERS
- PEPTOSTREPTOCOCCUS
- GRAM POSITIVE COCCI IN CHAINS
- BRAIN ABSCESS
- VEILLONELLA PARVULA
- GRAM NEGATIVE COCCI

CLOSTRIDIUM SPECIES

- LARGE GRAM POSITIVE RODS
- SPORE FORMATION
- Causative Agents For
 1. Gas gangrene : *Cl. perfringens* and other e.g septicum
 2. Tetanus : *Cl. tetani*
 3. Botulism : *Cl. botulinum*
 4. Toxic enterocolitis : *Cl. difficile* (Pseudomembranous colitis)

Clostridium perfringens (Cl. welchii)

- Morphology large rods gram +ve with bulging endospores
- 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

Clostridium perfringens (Cl. welchii)

Can leads to the following diseases

- 1) Wound Contamination
- 2) Wound infection
- 3) Gas Gangrene ~ most important disease
- 4) Gas Gangrene of the uterus in criminal abortion
- 5) Food Poisoning : Spores are swallowed → Germinate in gut after 18 hours (Toxin production) → 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)
- Prevention and Treatment
- Remove dead tissue , debris and foreign bodies .Penicillin and hyperbaric oxygen in some cases

Cl.tetani (TETANUS)

- Morphology gram +ve anaerobic with terminal spore. Drum Stick appearance
- Lives in soil and animal feaces. e,g horse and any wound can infected if contaminated by spores
- Face & neck wounds are more dangerous

Cl.tetani (TETANUS)

- Clinical Features
- Incubation period 1-3 weeks (time from infection to the appearance of symptoms)
- 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 Sardonius** (facial muscle)
- **Araching of Back** ~ strychnine
- **Opisthotonus** in children. *Opistho* meaning "behind" and *tonos* meaning "tension", due to **extrapyramidal effect and is caused by spasm of the axial along the spinal column** .

Cl.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.

Diagnosis

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

Cl.tetani (TETANUS)

Prevention

- by vaccination

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 .like penicillin. Supportive treatment by keeping the patient in dark pace, fluids and sedative valium

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

Symptoms

- 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 week* child, cranial nerve and constipation

Botulism Patogenesis

- 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 °C

Clostridium Difficile

Normal flora in gastrointestinal tract after exposure to antibiotics and killing of other normal flora, this organism will multiply and then produce toxin that has two components

- A-Subunit enterotoxin (cause diarrhea)
- B-Subunit Cytotoxic (kill the cells ie necrosis)

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

Severe dehydration , intestinal obstruction and perforation are some of complications of this syndrome

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)

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..

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

THANK YOU

(Foundation Block, Microbiology)

Dr. Ali Sornily &

Dr. Fawzia Al-otaibi