Microbiology Team
Med441

MED441
KING SAUD UNIVERSITY

Anaerobic Bacteria

Microbiology Team441

Editing File

Color Index:
- Main text
- Boys slides only
- Girls slides only
- **Doctor's notes**
- Extra information
- ***Important***

Objectives

- 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.
- Recognize specimens that are acceptable and unacceptable for anaerobic culture.

Objectives

- 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 OF ANAEROBES

- SPORE FORMING (only gram positive)
  - Gram positive (BACILLI)
    - Clostridia
      - Perfringens
      - Tetani
      - Botulinum
      - Difficile
- NON SPORE FORMING
  - Gram positive
    - Bacilli
      - Actinomyces
    - Cocci
      - Staphylococcus (peptococcus)
      - Streptococcus (peptostreptococcus)
  - Gram negative
    - Bacilli
      - Bacteroides
    - Cocci
      - Diplc cocci (veillonella)

Anaerobiosis

Why are they called anaerobiosis? Because they lack cytochrome

- Anaerobic bacteria 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 *(its mean the environment)*

These organism are normal flora in:

01

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: Bacteroides fragilis **and** Bifidobacterium species

02

Oropharynx
Examples:
1- Provetella melaninogenicus
2- Fusobacteria
3- Veillonella

03

Female genital tract (mainly in the vagina)

FEATURES OF ANAEROBIC INFECTIONS

Infections are always near to the site of the body which are habitat.
(In deep injuries and lack of oxygen **E.g.** atherosclerosis, diabetes, hypertension)

- Infection from animal bites. (Bc of normal flora in the animal mouth)
- Deep abscesses
- The infections are also **polymicrobial** (half anaerobic and half aerobic)
- Gas formation (seen in x-ray), foul smell
- Detection of "Sulphur granules"' due to actinomycosis
- Failure to grow organism from pus if not culture anaerobically.
- Failure to respond to usual antibiotics.

All anaerobic organisms that exist as normal flora in the upper part of the body “above the waist ” usually responses to penicillin.
And others that below the waist are penicillin resistance.

DISRUPTION OF BARRIERS:

- TRAUMA
- OPERATIONS (surgery)
- CANCEROUS INVASION OF TISSUES

How does the infection begin?

DISRUPTION OF BLOOD SUPPLY

- DROPS IN OXYGEN CONTENT OF TISSUE
- DECREASE IN Eh POTENTIAL (is a measurement use to detect oxygen reduction)
- TISSUE NECROSIS

WHAT ARE THE INFECTION CAUSED BY THESE ANAEROBIC?

- Post operative wound infection.
- Brain, dental, lung abscess.
- Intra abdominal abscess, appendicitis(التهاب الزائدة الدودية), diverculitis (انسدادات).
- Infection of the female genital tract: Septic abortion (infection of placenta and fetus), puerperal infection and endometritis , pelvic abscess or breast abscess.
- Diabetic foot infections and pilonidal sinus.

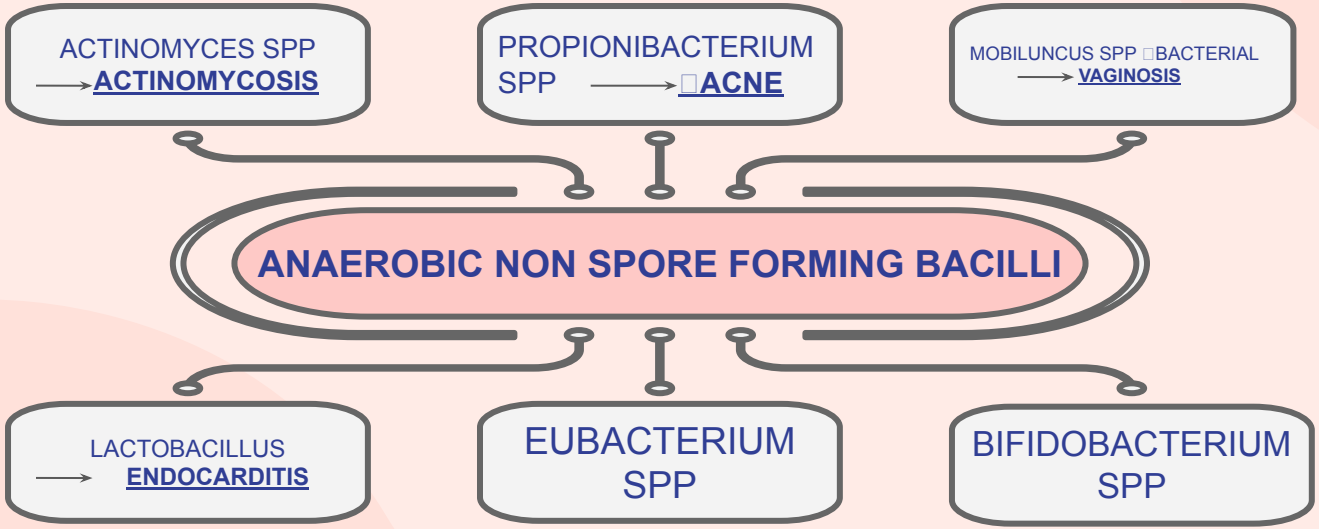
LABORATORY DIAGNOSIS:

When anaerobic infection is suspected:

- Specimens have to be collected from the site containing necrotic tissue
- Pus is better than swabs.
- Specimens has to be send to the laboratory within 1/2 hour. Why? So the organism won't die
- Fluid media like cooked meat broth are the best culture media.
- Specimens have to incubated anaerobically for 48 hours.

Treatment:

- Bacteroides fragilis is **always resistant to penicillin.** (So we don't use penicillin)
- But penicillin can be used for other anaerobes.
- Flagyl (metronidazole) is the drug of choice.
- Clindamycin can also be used.
- It has good coverage of anaerobic so it's useful in polymicrobial.
- Very good in streptococcus & staphylococcus coverage too.



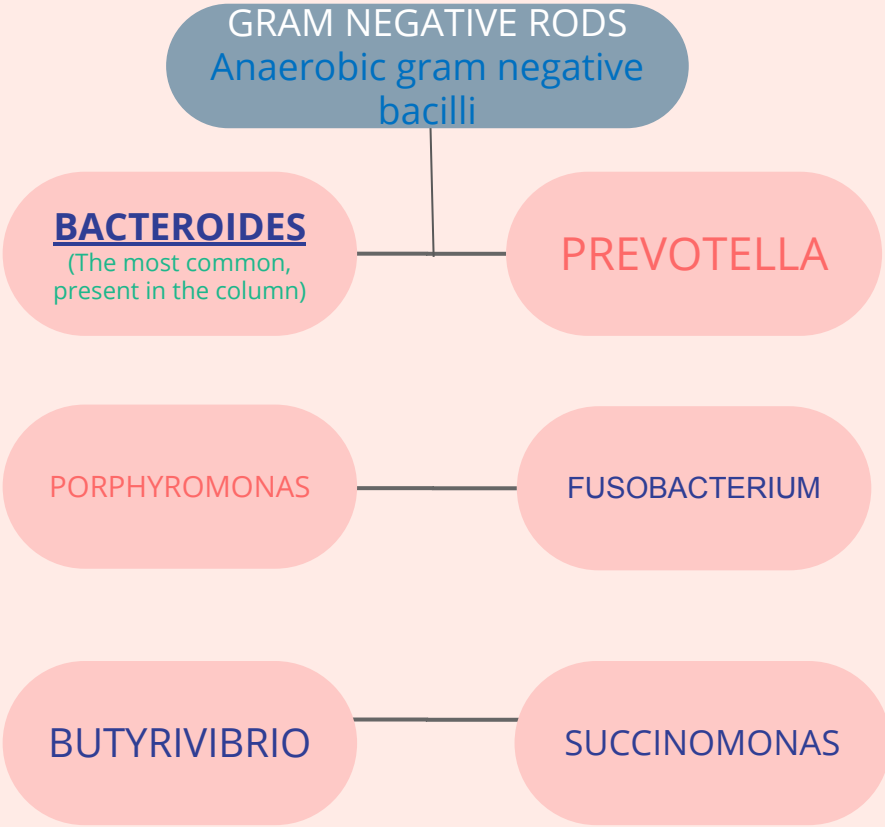
439 NOTES:

1-Q about classification: **branching** non-spore gram positive bacilli.
2-Q about Treatment: penicillin.
3-Case Q (e.g. patient had tooth extraction, mandibular abscess): actinomycosis
4-Q about shape of colony: molar tooth
5-Q about sulphur granules
actinomycosis اول ماتشوفون mandible على طول

ACTINOMYCOSIS *(the name of the disease)* (the most important one)

- Actinomyces(the name of the bacteria) 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:



BACTEROIDES:

- STRICT ANAEROBE
- PLEOMORPHIC
- GRAM NEGATIVE BACILLI (COCCO BACILLI)
- **NORMAL FLORA IN:**

1. OROPHARYNX
2. GASTROINTESTINAL TRACT
3. VAGINA

BACTEROIDES.FRAGILIS Group (B.FRAGILIS - B.VULGARIS - B.THETAIOTAMICRON - B.UNIFORMIS)	**BACTEROIDES SPECIES OTHER THAN B. FRAGILIS GROUP (PREVOTELLA - PORPHYROMONAS)**
ACCOUNT FOR 1/3 OF ALL ISOLATES (**most common**)	LESS COMMON, FOUND IN ORAL CAVITY
RESISTANT TO 20% BILE	BILE SENSITIVE
NO PIGMENTATION OF COLONIES OR FLUORESCENCE	SOME PIGMENTED
RESISTANT TO: **PENICILLIN, KANAMYCIN, VANCOMYCIN, COLISTIN –** AND MANY MORE	RESISTANCE ONLY TO **KANAMYCIN**

Other gram negative rods

- GRAM NEGATIVE BACILLI
- PERITONISILLAR → INTRNAL JUGULAR VEIN
- THROMBOSIS → EMBOLI TO THE LUNG

- GRAM POSITIVE COCCI IN CLUSTERS

- GRAM POSITIVE COCCI IN CHAINS
- BRAIN ABSCESS

- GRAM NEGATIVE COCCI
- SHEET-LIKE-or pairs

CLOSTRIDIUM SPECIES

01

Large Gram positive Rods

02

Spore Formation

03

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 (Pseudomembernous colitis)

Clostridium perfringens (Cl . welchii)

1- Morphology:

- Large rods (bacilli) Gram Positive.
- bulging Subterminal endospores

2- phatogenesis

- 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

3- 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

4-clinical features/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 swallowedGerminate in gut after 18 hours(Toxin production)abdominal pain and diarrhoea

5-prevention and treatment

- Surgical treatment: Remove dead tissue , debris and foreign bodies .
- Penicillin and hyperbaric oxygen in some cases

Cl.tetani (TETANUS)

1- 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

2-Laboratory diagnosis

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

3- phatogenesis

- 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.

4-clinical features/diseases

- 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 Sardonicus (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 .

5-prevention and treatment

1)prevention

- By vaccination

2)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 BOTULINUIM

- Toxin is exotoxin (protein) heat labile at 100 OC 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

1-origin

- Found in soil ponds and lakes
- From canned food., sea food e_g. salmon when it is not well cooked (Spores resist heat at 100 oC)then multiply and produce toxin

2-pathogensis

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

3-laboratory diagnosis

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

4-clinical features /diseases

- 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. .Childpresent with week child, cranial nerve and constipation

5-prevention and treatment

- Treatment: Mainly supportive and horse antitoxin in sever cases
- Prevention:Adequate pressure cooking autoclaving and heating of food for 10 minutes at 100 OC

Clostridium Difficile

1- Origin

Normal flora GIT

2- phatogenesis

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

1- A–Subunit enterotoxin (cause diarrhea)

2-Subunit Cytotoxic (kill the cells ie. necrosis).

3- 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

4-clinical features/diseases

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

5-prevention and treatment

- **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.

MCQs

Bacteroides fragillis is always resistant to ?			
A. Colistin	B. Kanamycin	C. Vancomycin	D. Penicillin
The organism which cause Gas gangrene is ?			
A. Cl.tetani	B. Cl.perfringens	C. Cl.difficile	D. Cl.botulinum
Morphological large rods gram positive with terminal spore ?			
A. Cl.welchii	B. Cl.botulinum	C. Cl.tetani	D. Actinomycetes spp
Non spore forming gram negative cocci ?			
A. Diplococci	B. Peptococcus	C. Peptostreptococcus	D. Actinomyces
The organism which diagnosis by gram stain with sulfur granules and growth of molar tooth shaped colonies?			
A. Mobiluncus spp	B. Lactobacillus spp	C. Actinomycosis	D. Eubacterium spp

1.D 2.B 3.C 4.A 5.C

SAQ

1. When anaerobic infection is suspected what we do? List 3.

Slide 9.

2. Why anaerobic bacteria cannot use oxygen as hydrogen acceptor?

Slide 5.

3. List 4 features of anaerobic infections:

Slide 6.

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