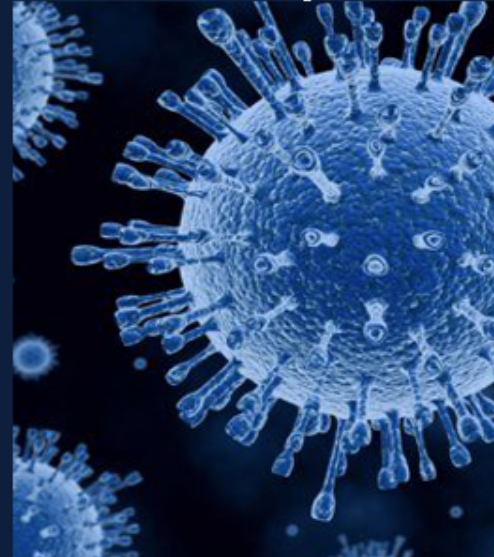
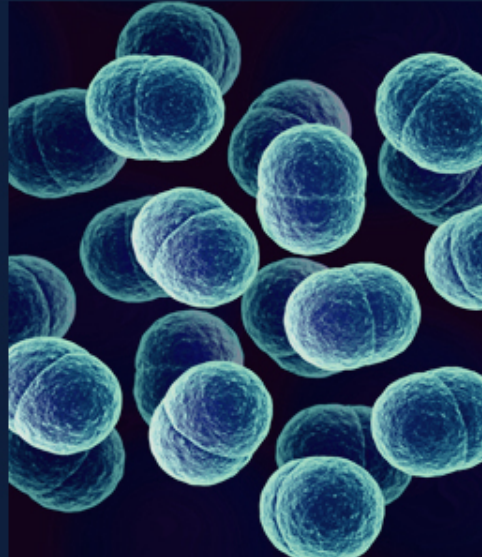


MICRObiology

TEAM 435

هذا العمل لا يغني عن المرجع الأساسي للمذاكرة



Lecture 9 Anaerobic Bacteria

● Important

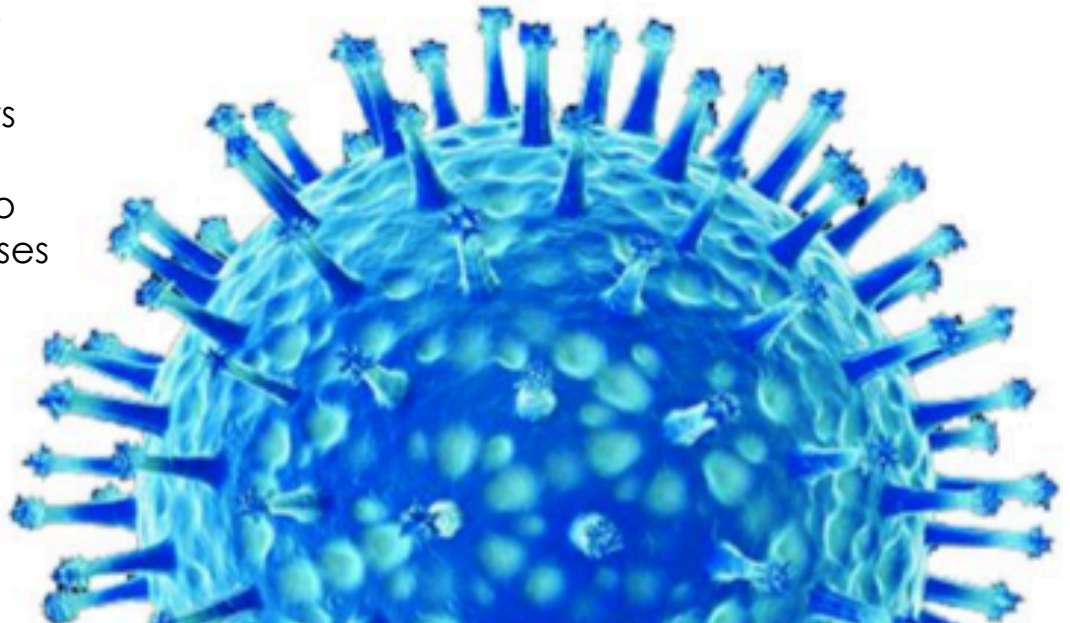
● Term

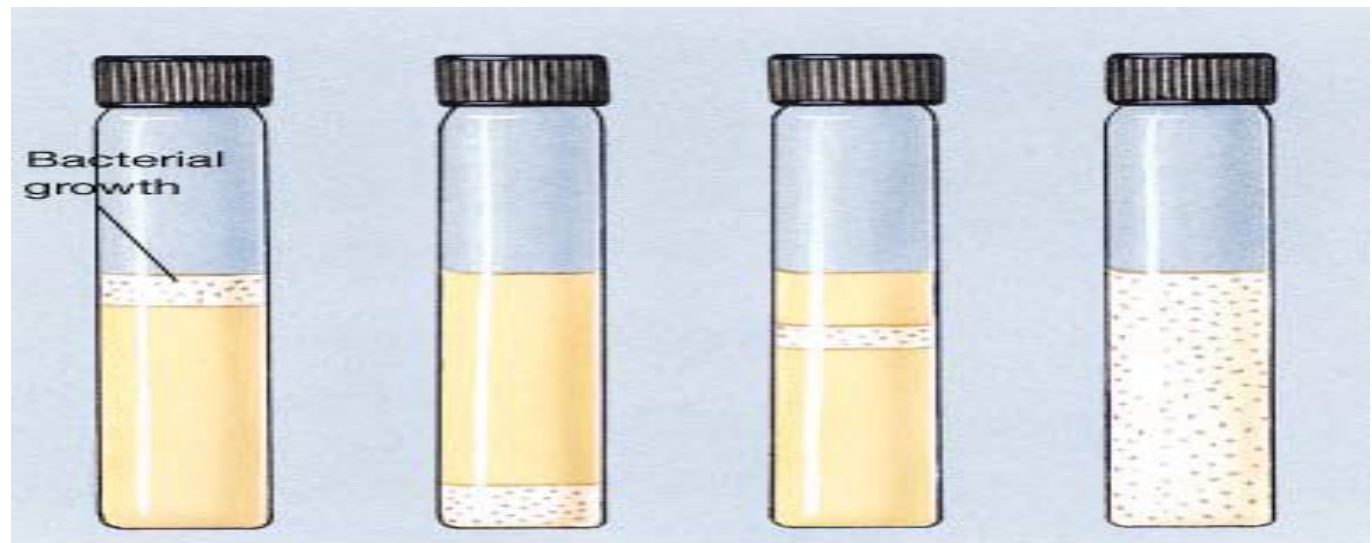
● Extra explanation

● Additional notes

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, Facultative anaerobes and aerotolerant 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.
- 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.





Bacterial growth

Obligate aerobe

Obligate anaerobe

Microaerophile

Facultative anaerobe



Need oxygen because they cannot ferment or respire anaerobically. They gather at the top of the tube where the oxygen concentration is **highest**.

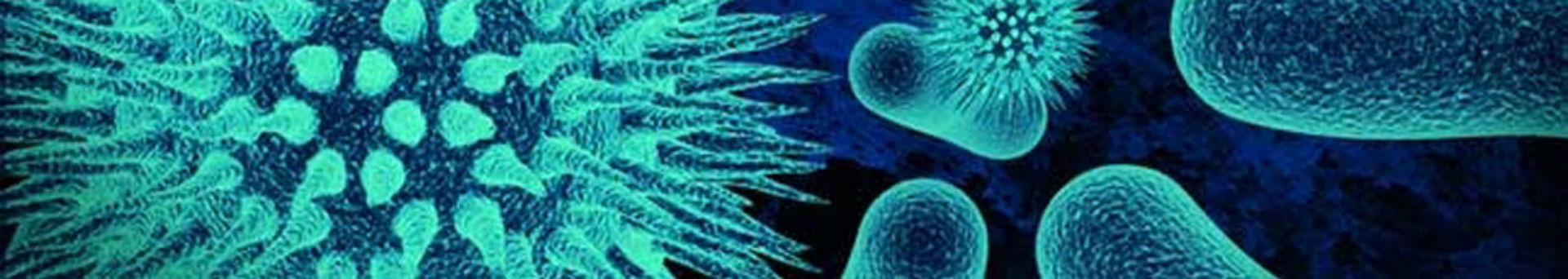
Are poisoned by oxygen, so they gather at the bottom of the tube where the oxygen concentration is **lowest**.

Need oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen. They gather in the upper part of the test tube but not the very top.

An organism that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent.

Why can't anaerobic bacteria survive in oxygen?

The presence of oxygen leads to the production in cells of the **superoxide radical** (a negatively charged O_2 molecule). Normally, the superoxide anion is lethal enough to kill almost any organism. Aerobic organisms and facultative anaerobes have the **enzymes superoxide dismutase and catalase**. These enzymes work together to convert superoxide to oxygen and hydrogen peroxide.



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: Normal Flora in...

Oropharynx

- *Provetella melaninogenicus*
- Fusobacteria
- Veillonella

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) *B acteroides fragilis* (2) *Bifidobacterium species*

Female genital tract

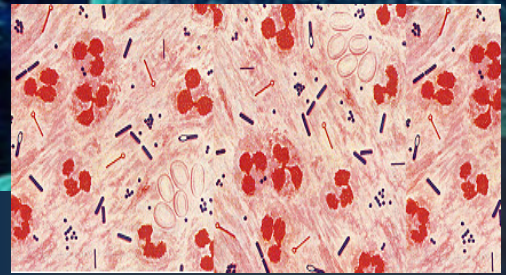
- Mainly in the vagina

+Skin, Nose, Endocervix and Urethra

MODIFIED BY

- Pathophysiologic states
- Antimicrobial agents, H-Blockers, antacids
- Hormonal changes
- Age

Classification



A. NON SPORE FORMING {MORE COMMON}

GRAM POSITIVE BACILLI Actinomyces

- *Propionobacterium propionicum*
- *p.acne*
- *Bifidobacterium*
- *LAEuobacterium*
- *CTOBACILLUS*
- ***Actinomyces israelii***

GRAM POSITIVE COCCI

- *Peptococci* (***staphylococcus***)
- *Peptostreptococci* (***streptococci***)

GRAM NEGATIVE BACILLI (cocco bacilli) Bacteroides

- ***Bacteroides fragilis*** (*resistant to penicillin*)
- *Prevotella spp*
- *Leptotricha buccalis*
- ***fusobacterium spp*** (The main cause of pharyngitis)
f.nucleatum

GRAM NEGATIVE COCCI

- *Viellonella sp.* (**diplococci**)

B.SPORE FORMING

GRAM POSITIVE BACILLI Clostridia

- **CL** .*perfringens*
- **CL** .*Septicum*
- **CL** .*novyi*
- **CL** .*Histolyticum*
- **CL** .*Difficile*
- **CL** .*Tetani*
- **CL** .*Botulinum*

Importance

- Dominate the indigenous flora (colonization resistance)
- **Commonly found in infection**
- Easy to overlook
- Special precautions-
- Slow growth
- Mixed infection
- Difficult treatment

• Account for 1/3 of all isolates

• **Resistant** to 20% bile

• Resistant to many antibiotics~*

Penicillin, kanamycin, vancomycin, colistin...etc

• **No pigmentation** of colonies or fluorescence

* **Flagyl** (metronidazole) is the drug of choice. **Clindamycin** can also be used.

Bacteroides:

1.B. Fragilis Group

2.B.Vulgaris

3.B.Thetaiotamicron

4. B.Uniformis

Bacteroides species other than B. Fragilis group

• Bile sensitive

• Resistant to **kanamycin only**

• Some pigmented

Features of anaerobic infections

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

* Infection from animal bites.

* Deep abscesses. (خُرَاج)

* The infections are also polymicrobial (caused by several types of microorganisms)

* Gas formation, 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.

* Suppuration

* Tissue destruction {gangrene}

* Septic thrombophlebitis

• Some have unique pathology: (Actinomycosis, Pseudomembranous colitis, Gas gangrene)



Sulphur granules

One of the small yellow bodies found in the pus of actinomycotic abscesses and consisting of clumps of the causative actinomycete

What are the infection caused by these anaerobic organisms ?

* Post operative wound infection

* Brain abscess

* Dental abscesses

* Lung abscess

* Intra-abdominal abscess, appendicitis, diverticulitis

* All these infection can cause bacteremia

* Infection of the female genital tract

* Septic abortion

* Puerperal infection or sepsis

* Endometritis

* Pelvic abscess or other abscess

* Other infections

a) Breast abscess in puerperal sepsis

b) Infection of diabetic patients (diabetic foot infections).

c) Infection of pilonidal sinus

EPIDEMIOLOGY

Almost all infections are indigenous except

- *Tetanus (الكزاز - تشنج العضلات)
- *Infant ,wound **botulism** (تسمم من أكل اللحوم)
- * Gas gangrene { some cases }
- * Bites
- * C .difficile {nosocomial (**acquired or occurring in a hospital**) }

PREDISPOSING FACTORS:

- Low O tension {Eh}
- Trauma, dead tissue , deep wound
- Impaired blood supply
- Presence of other organisms
- Foreign bodies
- Antibiotic therapy
- Neoplasm
- Trauma
- Cholecystitis
- Obstruction
- Ulceration
- Diabetes mellitus
- Pylephlebitis
- Diverticula formation

How does Infections begin?

Disruption of barriers

- ❖ Trauma
- ❖ Operations
- ❖ Cancerous invasion of tissues

Disruption of blood supply

- ❖ Drops oxygen content of tissue
- ❖ Decrease in Eh potential
(**redox potential**)
- ❖ Tissue necrosis

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.
- d) Fluid media like cooked meat broth are the best culture media.
- e) Specimens have to incubated anaerobically for 48 hours.



ACTINOMYCOSIS

Actinomyces are branching anaerobic or microaerophilic Gram positive bacilli.

Source of the infection: normal flora and the host usually normal host.

Primary site of the infection: mouth, lung, appendix, uterus with **IUD*** (chronic infection)

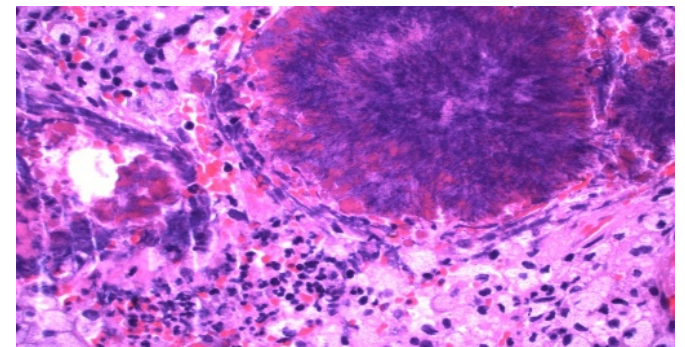
Infection can spread to: brain, liver, bone and blood.

•**Diagnosis:** by Gram stain with sulfur granules and growth of molar tooth colonies.

•**Treatment:** penicillin, clindamycin or tetracycline.



An intrauterine device (IUD or coil) is a small contraceptive device, often 'T'-shaped, often containing either copper or levonorgestrel, which is inserted into the uterus. They are one form of long-acting reversible contraception which are the most effective types of reversible birth control.



Clostridium perfringens (CI . 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

Can leads to the following diseases

- Wound Contamination
- Wound infection
- **Gas Gangrene** - most important disease
- Gas Gangrene of the uterus in criminal abortion
- Food Poisoning : Spores are swallowed → Germinate in gut after 18 hours (Toxin production) → abdominal pain and diarrhoea

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

Clinical Features

Incubation period 1-2 weeks (time from infection to the appearance of symptoms)

Symptoms:

Painful muscle spasm around infected wound and Contraction of muscles in the face called **Trismus** (Lockjaw) , **Risus Sardonicus** - strychnine or back called araching of Back

- 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 .**

Pathogenesis

Mainly due to **tetanospasmin** which is powerful exotoxin (protein) .This organism does not lead to invasion or Bacteraemia . Its function to inhibit 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.

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 place, 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.

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 weak child, cranial nerve and constipation

Botulism Pathogenesis

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

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 severe 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 with then produce toxin that has two components:

- a. Subunit enterotoxin (cause diarrhea)
- b. Subunit Cytotoxic (kill the cells i.e. necrosis)

* Pseudomembrane 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 complication of this syndrome

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)

Treatment:

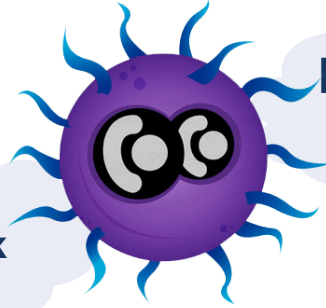
Metronidazole or and oral vancomycin in severe cases

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.



Online Quiz



Pull my
pili!

Fine!
Just click
[HERE](#)



Videos

You should watch
these videos. They are
very helpful

<https://www.youtube.com/watch?v=kg3U5PxdeXw>

<https://www.youtube.com/watch?v=fjbxFAC-v3Y>

<https://www.youtube.com/watch?v=67-YySMQqQc>

<https://www.youtube.com/watch?v=FW6xA46UB3o>



Books that could help you

Lippincott's Illustrated Reviews



MICRObiology

TEAM 435

We do things better

Boys Team

- Ali Alzahrani
- Khalid Sharahily
- Ahmad Alzahrani
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- Muhammad Dossary
- Meshal Alhazmy
- Hamzah Alfiar

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- Dalal Alhuzaimi
- Reema Allhaidan

Girls power!



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