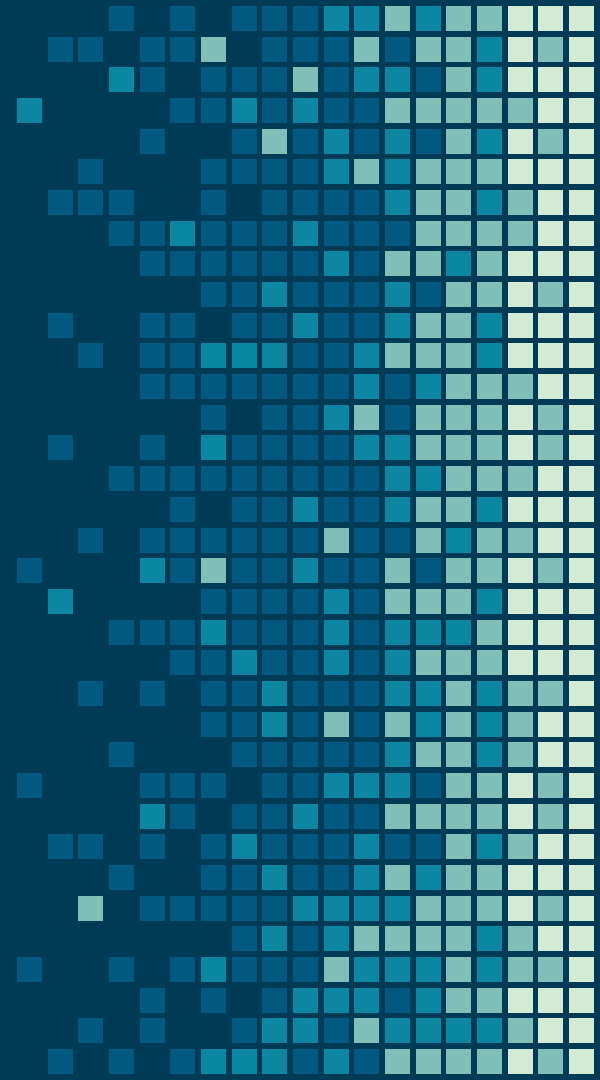
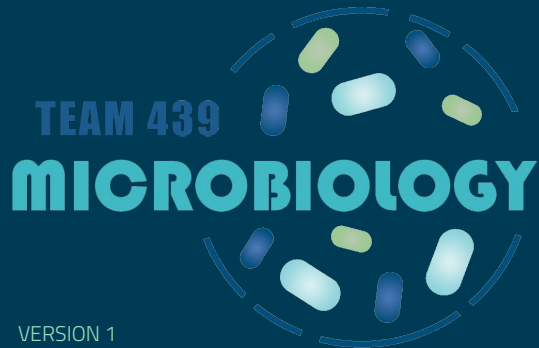


# Anaerobic Bacteria



# Objectives

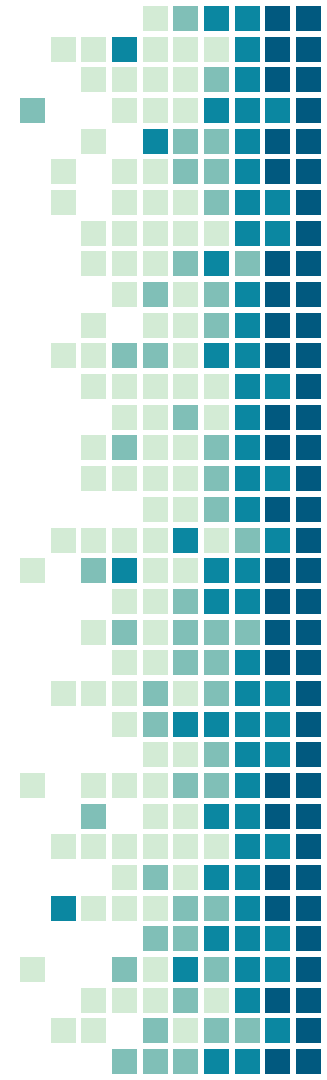
- ❖ Describe anaerobic bacteria including their sensitivity to oxygen and where they may be found in the environment and the human body.
- ❖ Differentiate the various types of anaerobes with regard to atmospheric requirement (i.e. obligate anaerobes, Facultative anaerobes and aerotolerant anaerobes.
- ❖ Describe how anaerobes, as a 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.

## Colour index:

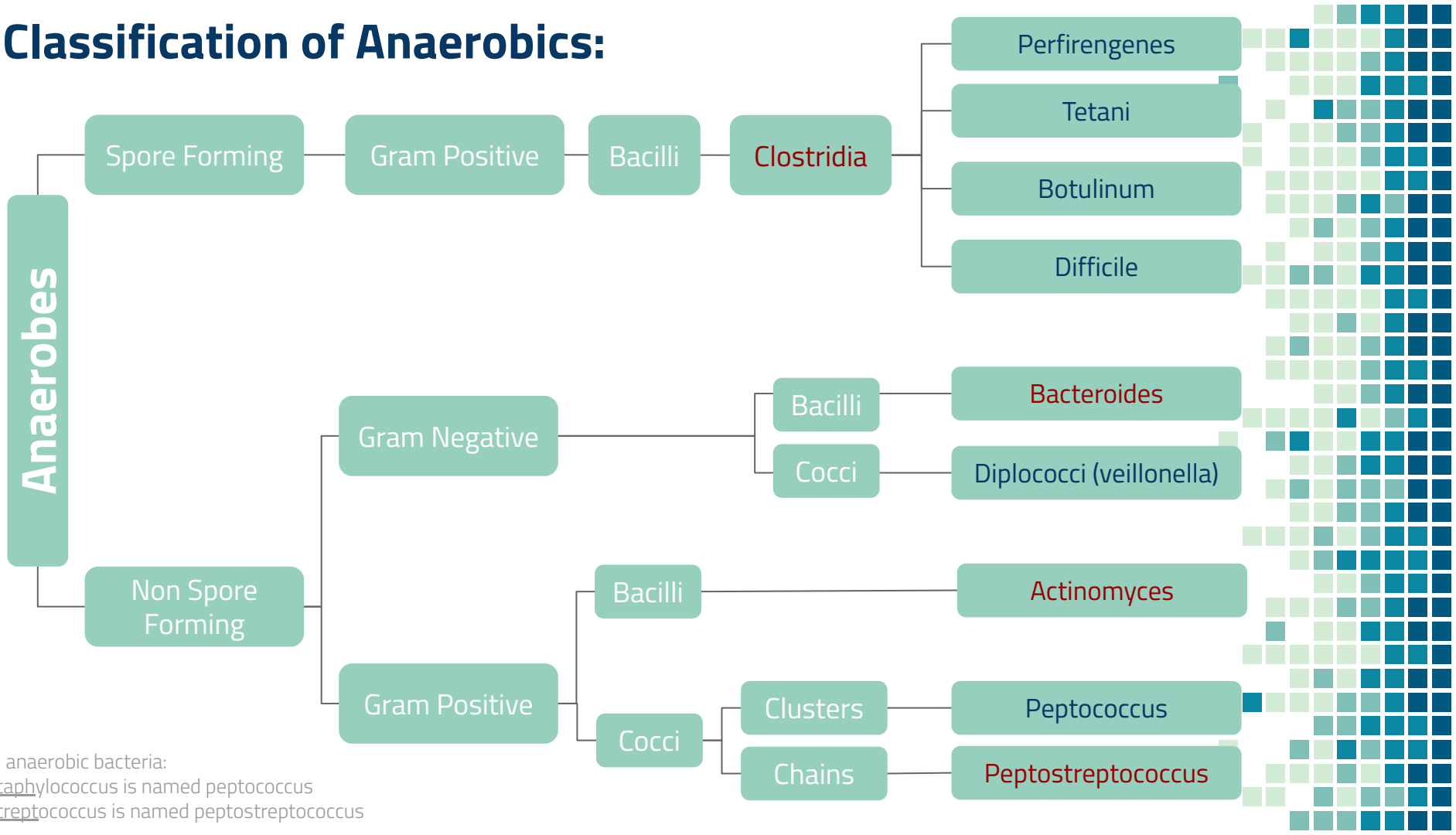
- **Red: Important.**
- Grey: Extra info & explanation.
- Purple: only in girl's slides.
- Green: Only in boy's slides.
- 

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will be in the editing  
file, so please check it  
frequently.

Scan the code  
Or click [here](#)



# Classification of Anaerobics:



In anaerobic bacteria:

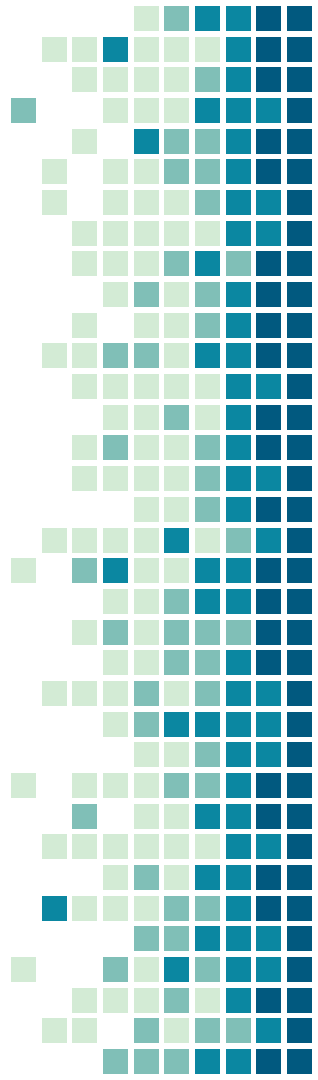
Staphlococcus is named peptococcus

Streptococcus is named peptostreptococcus

# Anaerobiosis

- ❖ Anaerobic bacteria lack cytochrome, so they **cannot** use oxygen as hydrogen acceptor. (Most lack **Catalase** and **Peroxidase**). They cannot grow in the presence of oxygen, it is toxic to them. WHY? Because they don't have the enzymes (cytochrome oxidase, catalase and peroxidase) to eliminate reactive oxygen species.
- ❖ Contain **flavoprotein**, so in the presence of oxygen they produce  $H_2O_2$  (hydrogen peroxide), which is toxic.
- ❖ Some lack enzyme **superoxide dismutase**, so they can be easily killed, peroxide and toxic radicals shuts enzymes like fumarate reductase (must be in reduced form to work).

Lacking superoxide dismutase allows low oxygen radicals to accumulate in anaerobic bacteria which leads to damage, not just that, but this accumulation will also inactivate other bacterial essential enzyme systems that only works under low or no oxygen conditions such as fumarate reductase.



# Habitat I :

1

**Oropharynx** (between the pharynx and the mouth)

**E.g. :**

- Prevotella melaninogenica
- Fusobacteria
- Veillonella.

2

**Gastrointestinal tract**

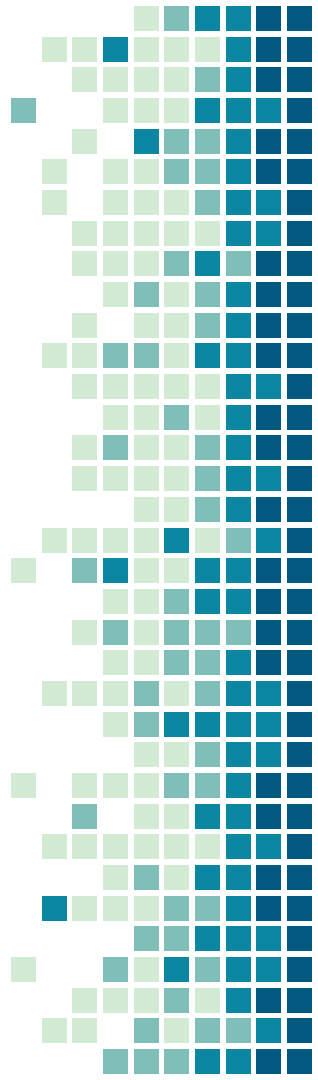
Found mainly in the large **colon** in large numbers  
Total number of anaerobes =  $10^{11}$   
While all aerobes (including E. coli) =  $10^{14}$   
examples are Bacteroides fragilis,  
Bifidobacterium species.

3

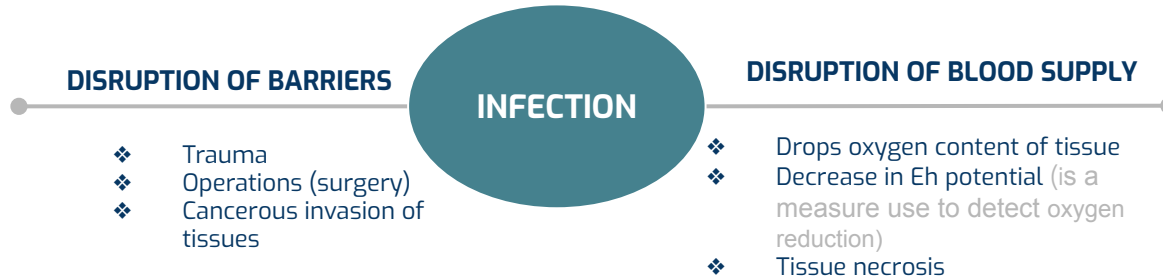
**Female genital tract  
(mainly in the vagina)**

## Features of Anaerobic Infections:

- ❖ Infection from animal bites.
- ❖ Deep abscesses.  
(collection of pus within the tissue of the body )
- ❖ The infections are also polymicrobial.  
(caused by several types of microorganisms)
- ❖ Gas formation, foul smell.
- ❖ Detection of "**Sulphur granules**"\* due to actinomycosis.  
(\*small yellow bodies found in the pus of actinomycotic abscesses)
- ❖ Failure to grow organism from pus if it is not cultured anaerobically. (without oxygen)
- ❖ Failure to respond to usual antibiotics.

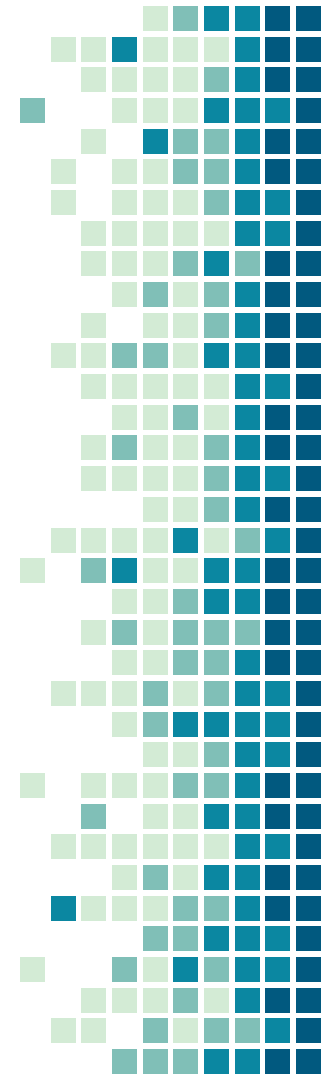


# How Does the Infection Begin?



## Infections Caused by Anaerobic Organisms

- ❖ Post operative wound infection.
- ❖ Brain, dental, lung abscess.
- ❖ Intra abdominal abscess, appendicitis (التهاب الزائدة الدودية), diverticulitis (انسدادات).
- ❖ Infection of the female genital tract: Septic abortion (Infection of the placenta and fetus), puerperal infection and endometritis, pelvic abscess or breast abscess.
- ❖ Diabetic foot infections and pilonidal sinus. (Diabetic foot that exhibits any pathology resulting from diabetes mellitus)



# 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? Because they will convert  $O_2$  to  $H_2O_2$  (Toxic). Then, they will die.

Fluid media like cooked meat broth are the best culture media.

Why? Because it contains nutrients & proteins, which can help the anaerobic bacteria to grow.

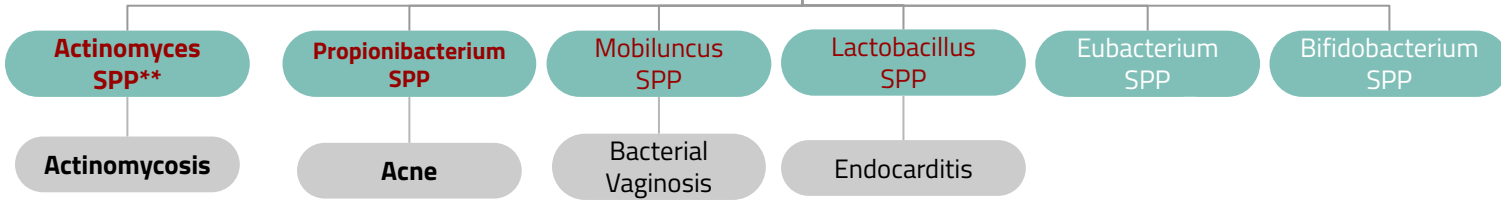
Specimens have to incubated anaerobically for 48 hours.

# Treatment

- ❖ **Bacteroides fragilis** is **always** resistant to **penicillin**.
- ❖ But penicillin can be used for other anaerobes.
- ❖ Flagyl (metronidazole) is the drug of choice. (The favored pharmaceutical treatment for doctors to prescribe)
- ❖ Clindamycin (antibiotic) can also be used.



## ANAEROBIC NON-SPORE FORMING BACILLI

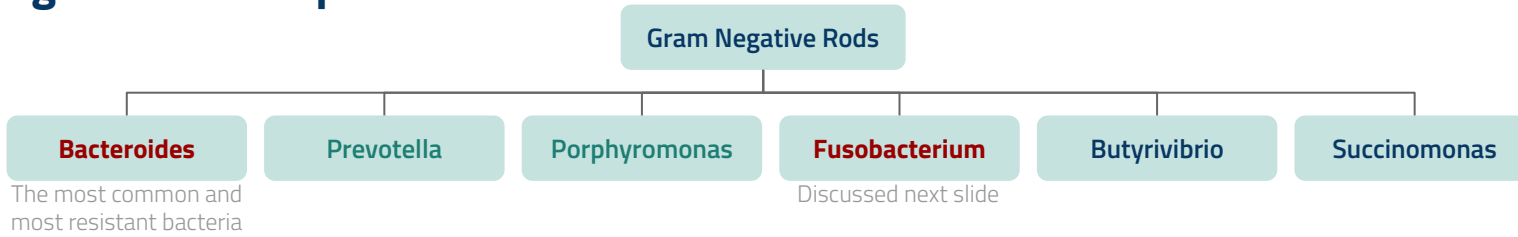


# Actinomycosis

- ❖ **Classification:** Actinomyces are **branching**, anaerobic or microaerophilic, non-spore forming Gram positive bacilli
- ❖ **Source of the infection:** is **Normal flora** (e.g. of oral cavity) and the host usually normal host.
- ❖ **Primary site of the infection:** is mouth (dentally), lung, appendix, uterus with IUD (**chronic infection**). IUD = اللولب لمنع الحمل
- ❖ **Infection can spread to:** the brain, liver, bone and blood.  
Because it is close to the source of infection (of normal flora e.g. oral cavity).
- ❖ **Diagnosis:** by Gram stain with **sulfur granules** and growth of **molar tooth shaped colonies**.  
(the colonies looks like the shape of molar tooth, popcorn like)
- ❖ **Treatment:** **penicillin**, clindamycin or tetracycline (or surgical drainage of abscess)



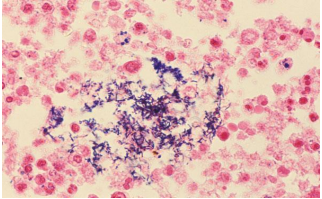
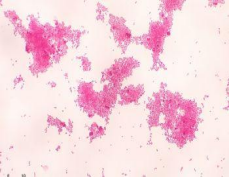
# Organisms Groups:



## Strict anaerobe, pleomorphic, gram negative bacilli (cocci/bacilli)

<b>BACTEROIDES FRAGILIS GROUP</b> Often associated with intra-abdominal & soft tissue infections <b>below the waist</b> تحت الخصر	<b>BACTEROIDES SPECIES OTHER THAN B.FRAGILIS GROUP</b> <b>Above the waist</b> فوق الخصر
Account for 1/3 of all isolates <b>(They Are more Common)</b> . Normal flora in GIT .	Less common. Normal flora in Oral cavity.
B. FRAGILIS - B. VULGARIS - B.THETA IOTAOMICRON - B. UNIFORMIS	PREVOTELLA - PORPHYROMONAS
<b>Resistant to 20% bile</b> (can live in GIT)	Bile sensitive
<b>More resistant to many antibiotics:</b> Penicillin, kanamycin, vancomycin, colistin, and many more. <b>(Resistant to penicillin because it produces beta-Lactamase)</b> (can be treated by Flagyl)	Resistant to Kanamycin only
<b>No pigmentation</b> of colonies or fluorescence (more resistant and more common to cause infection.)	Some pigmented



Other Anaerobic Gram <b>NEGATIVE</b> Bacilli	Anaerobic Gram <b>POSITIVE</b> Cocci		Anaerobic gram <b>NEGATIVE</b> Cocci
Bacilli	Chains	Clusters	Sheet-like
<b>Fusobacterium Necrophorum</b>	<b>Peptostreptococcus</b>	Peptococcus	Veillonella Parvula
Peritonsillar → Internal jugular vein thrombosis → Emboli to the lung (pulmonary infection) <b>Lemierre Syndrome</b>	<b>Associated Brain abscess</b> 	-	

Causes sore throat and radial abscess, which can spread to **jugular vein** and cause **thrombosis** that can affect the lungs and cause **pulmonary embolism (Lemierre Syndrome)**.

### Notes:

- Patient with jugular vein thrombosis. What's the organism?
- Patient with pulmonary embolism. What's the organism?
- Q about the classification, (gram -ve bacilli etc..)
- What is the organism that cause lemierre syndrome?



# CLOSTRIDIUM SPECIES

Clostridium is one type of bacteria, but it has species, and each one causes a different disease.

Causative agent هو العامل المميز بالفصيلة والتي يخليها تسبب مرض معين

- ❖ All members of Clostridium are large, spore forming, **Gram positive rods**.

S-T= Sub terminal T = terminal ليست اختصارات علمية فقط للتوضيح	Clostridium species	Causative Agent for " disease "	
S-T	Cl. <b>perfringens</b> and other e.g septicum	Gas gangrene	In human
T	Cl. <b>tetani</b>	Tetanus	In animals
S-T	Cl. <b>botulinum</b>	Botulism	In animals
S-T	Cl. <b>difficile</b> (Causes Pseudomembranous Colitis)	Toxic enterocolitis	In human

كلهم يشتغل عليهم الـ (Penicillin) بإستثناء (Cl. difficile) يشتغل عليها (Metronidazole/Flagyl)

# Cl. perfringens (Cl. welchii)



Scan or Click

## 1-Morphology:

- large rods (bacilli) Gram Positive.
- Bulging **Subterminal** endospore

## 2-Pathogenesis:

- Traumatic open **wounds** or compound **fractures** lead to **muscle damages** and (from food) **contamination with dirt** etc.
- Mainly in **war wounds**, old age, diabetic patients because of **low blood supply** and amputation of thigh (required prophylaxis with penicillin).

## 3-Laboratory diagnosis:

- Smear (**coat with**) Gram stain Large Gram positive bacilli with **few or no WBCs**
- Culture
- Blood agar with haemolytic colonies (**double zone of haemolysis** because they produce toxin called phospholipase).

It means when we put it in a bloody medium, we see 2 types of haemolysis around the colony

- Cooked meat medium
- **Gives the NAGLER'S Reaction & toxin neutralization** on Egg yolk medium & toxin is a phospholipase.

-Nagler's reaction is a biochemical test used to identify organisms which liberate phospholipases.

-Egg yolk agar is a medium used to differentiate Clostridium spp. and other obligate anaerobes.

## 4-Clinical Features/Diseases:

- 1-Wound Contamination that will lead to Wound infection, leads to **Gas Gangrene**, the most important disease.
- 2-Gas Gangrene of the uterus in criminal abortion
- 3-**Food Poisoning** : Spores are swallowed ⇒ Germinate in gut after 18 hours (Toxin production) ⇒ abdominal pain and diarrhea.

## 5-Treatment and Prevention:

- **surgical treatment:** Remove dead tissue, debris and foreign bodies.
- **Penicillin** and hyperbaric oxygen in some cases. اعطاء اوكسجين للمريض

# Cl. tetani

**Morphology:** large rods Gram Positive. With **Terminal spore**  
"Drumstick appearance"

## 1-Origin:

**in soil and animal feces. E.g horse** and any wound can be infected if contaminated by spores (Face & neck wounds are more dangerous)

## GIT of Animals

## 6-Prevention: by vaccination

## 2-Pathogenesis:

Mainly due to **tetanospasm** (powerful exotoxin -protein-). This organism does not lead to invasion or Bacteraemia (presence of bacteria in blood).

Its function: to inhibit transmission of **normal inhibitory messages from CNS** at anterior horn cells of cord.

## 5-Treatment:

Cleaning of wound and removal of Foreign body

- Specific by **antitoxin** from horse serum but it can lead to anaphylaxis & shock (so it must be tested first).
- Human **immunoglobulin (antibodies)**.
- Antibiotics: like **penicillin**.

Supportive treatment by keeping the patient in dark place, fluids and sedative valium.

## 3-Laboratory diagnosis:

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

## 4-Clinical Features/Diseases:

- 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 (mandible) in the face** called Trismus (Lockjaw).

Risus Sardonius: spasm of the facial muscles due to strychnine poisoning.

تشنج غير طبيعي يحدث في عضلات الوجه بحيث يظهر على شكل ابتسامة عريضة

- Opisthotonus: Arching of **Back** in children. (التشنج الظهري)

Opistho = behind, tonos = tension. due to extrapyramidal effect and is caused by spasm of the axial along the spinal column.



# Cl. botulinum

(Remember **botulism** is from bad **bottles** of food)

Toxin is **exotoxin** (protein) heat labile at 100°C and it resists gastrointestinal enzymes.

It is the **most powerful toxin known**  
Lethal dose 1 µg human and 3 kg kill **all population of the world**. It is dictated for by lysogenic phage.

## 2-Pathogenesis

Attacks **neuromuscular junctions** and prevents release of **acetylcholine** that can lead to flaccid **paralysis**

طريقته انه يهاجم الوصلات العصبية مثل اللي تربط العضلة بالعصب، وراح يمنع افراز النواقل العصبية مثل الاسيتل كولين، Ach فيالتالي بيحصل شلل.

## 1-origin:

**in soil ponds and lakes, canned food, seafood like salmon when it is poorly cooked or smoked.**

Spores resist heat at 100°C then they multiply & produce toxins.

## 3-Laboratory diagnosis:

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

In other words: they take this organism from human feces, then they culture it, after that they inject it to mice, the result is paralysis & death of the mice.

## 4-Clinical Features/Diseases:

-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** (تسمم حديثي الولادة): Ingestion of: Spores germination in the gut  
**Botulism** Child-present with week child, cranial nerve and constipation.

## 5-Treatment & Prevention:

**Treatment** :Mainly supportive therapy and horse antitoxin in severe cases

- **Penicillin**

**Prevention:** Adequate pressure cooking autoclaving and heating of food for 10 minutes at 100°C



Scan or Click

# Cl. Difficile

\*Inflammation of the large intestine (colon) due to an overgrowth of *Clostridium difficile* (*Cl difficile*) bacteria.

## 1-origin:

Normal flora in GIT

## 2-Pathogenesis:

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 i.e. necrosis).

## 5-Treatment & Prevention:

**Treatment:** Metronidazole or/and oral vancomycin in severe cases.

**Prevention:** This organism **form spores** and is hard to control in the hospital because they are **resistant to alcohol** decontamination (use Na hypochlorite instead).

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

## 3- Lab diagnosis

this organism is hard to grow in the laboratory, it requires special media and growth of the organism in solid media requires 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)**

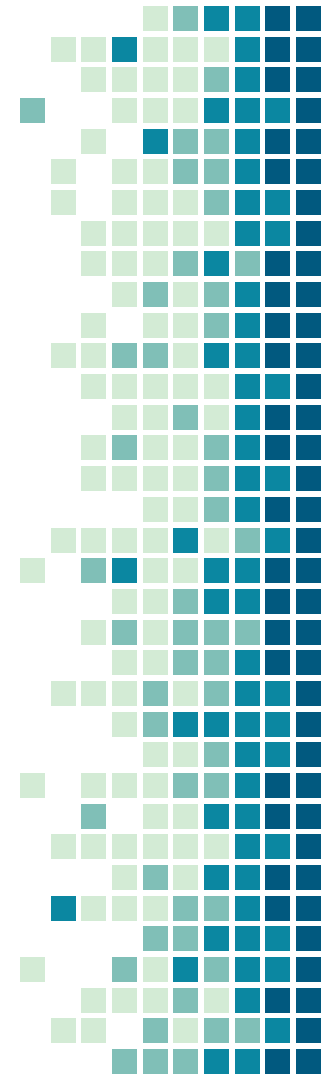
## 4-Clinical Features/Diseases:

- **PSEUDOMEMBRANOUS COLITIS\*** is the clinical manifestation of this disease which composed of bacteria , fibrin , WBCs and dead tissue cells (necrosis).
- **Severe dehydration** (due to Diarrhea), intestinal obstruction and perforation are some of complication of this syndrome.



# Summary for Clostridium Species

Click [HERE](#)  
Or Scan The Code





# Notes..

1-The presence of oxygen leads to the production of the superoxide radical (a negatively charged O<sub>2</sub> molecule). Normally, the superoxide anion is lethal enough to kill almost any organism, and **anaerobic bacteria lack the enzymes that degrade it.**

2-Anaerobic bacteria are also said to be **strictly anaerobic (obligate anaerobic)** to distinguish them from the **oxygen tolerant bacteria** and **facultatively anaerobic bacteria.**

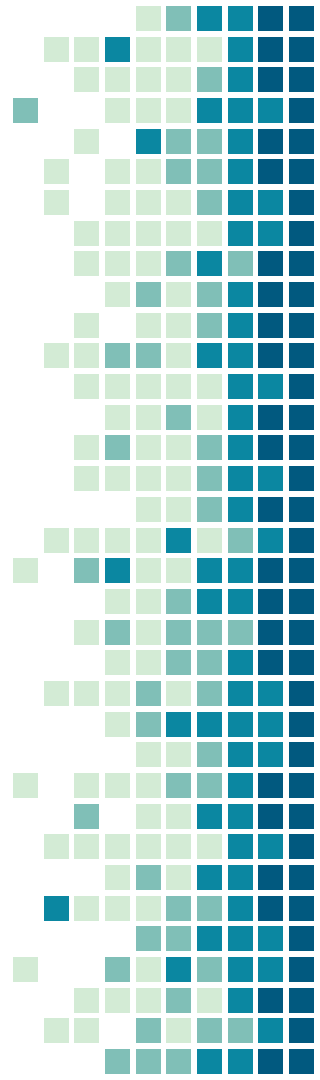
- Oxygen tolerant bacteria: does not use oxygen in their metabolism. However, they are not poisoned by oxygen.
- Facultatively anaerobic bacteria: are not poisoned by oxygen, and can switch their metabolism.

3-The most important anaerobic bacteria is Bacteroides fragilis (**B.Fragilis**) because it's the most common and the most resistance bacteria. (they are normal flora in GIT, (below the waist), and non-pigmented).

4- **Cl. perfringens** (releases the toxin phospholipase and cause gas gangrene), **Cl. botulinum** (Causes paralysis), **Cl. tetani** (Causes spasm), and **Cl. difficile** (causes dehydration due to diarrhea).

5- Cl. difficile releases two kinds of toxins, enterotoxin that causes diarrhea and cytotoxic that kills the cell.

6- Anaerobes in the oral cavity are more responsive & susceptible to penicillin than the ones below the waist or abdomen which are resistant.



# MCQs

**1- A scientist takes a sample from the bottom of a nearby lake. He divides the sample into three test tubes. In the first test tube, he adds an air pump. In the second test tube, he seals the cap and removes the oxygen. The third tube he leaves unsealed, and undisturbed. Which tubes will NOT grow obligate anaerobes?**

- A- Tubes 1 and 3
- B- Tube 1 only
- C- Tube 2 only
- D- Tube 3 only

**2- What is the organism that cause lemierre syndrome?**

- A- Peptococcus
- B- **Succinomonas**
- C- Peptostreptococcus
- D- Fusobacterium Necrophorum

**3- Gas gangrene is the causative agent for?**

- A- Cl. perfringens
- B- Cl. botulinum
- C- Cl. tetani
- D- Cl. difficile

**4- The organism that grows a molar tooth shaped colony is:**

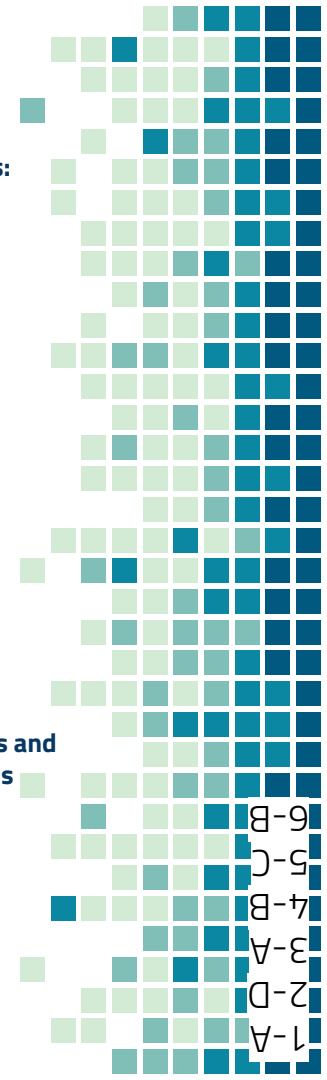
- A- **Porphyromonas**
- B- Actinomyces
- C- Peptostreptococcus
- D- Fusobacterium Necrophorum

**5- Organism that is usually associated with brain abscess?**

- A- Gram Positive Peptococcus
- B- Gram Negative Peptococcus
- C- Gram Positive Peptostreptococcus
- D- Gram Negative Peptostreptococcus

**6- Clostridium specie that attacks neuromuscular junctions and prevents release of acetylcholine that can leads to paralysis**

- A- Cl. perfringens
- B- Cl. botulinum
- C- Cl. tetani
- D- Cl. difficile



6-9  
5-5  
4-4  
3-A  
2-D  
1-A

# MCQs

**7- Anaerobic bacteria lack an enzyme called:**

- A- Lactase
- B- Superoxide dismutase
- C- Lyase
- D- Beta dismutase

**8- Treatment of most anaerobic bacteria is:**

- A- Penicillin
- B- Metronidazole
- C- Vancomycin
- D- Lamivudine

**9- Actinomyces cause actinomycosis. Actinomycosis is a chronic granulomatous infection that causes development of sinus tract, fistulae, which come to the surface and drain pus containing ..... Granules.**

- A- Zinc
- B- Iron
- C- Sulphur
- D- Iodine

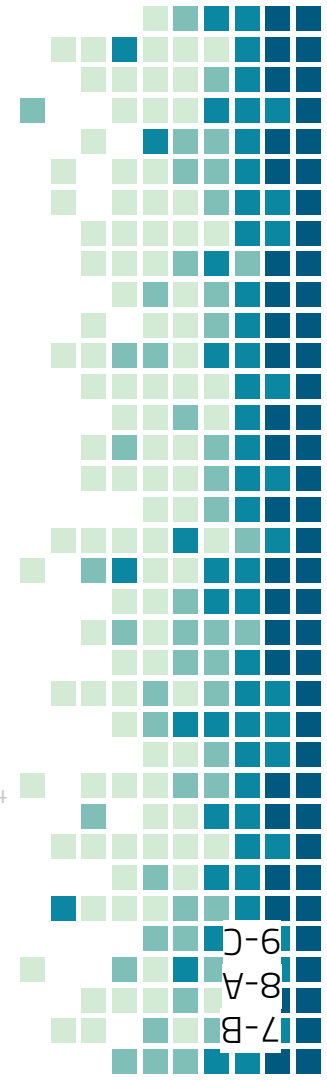
# SAQ

**1-What factors predispose patients to anaerobic infections?** Slide 5

**2-Why are anaerobes sensitive to O<sub>2</sub> intermediates?**

They have little superoxide dismutase to remove O<sub>2</sub> radicals, low amount of catalase to remove H<sub>2</sub>O<sub>2</sub> and often lack cytochromes.

**3-What bacteria is always resistant to penicillin?** Slide 4



9-C  
8-A  
7-B

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