

# Anaerobic Bacteria

— -Important

-In boy's slides

-Extra

-Notes

— -In girl's slides

In this link, you will find any correction or notes unmentioned in the team's work. Please check the link below [Frequently](https://docs.google.com/document/d/1WvdeC1atp7J-ZKWOUkSLsEcosjZ0AqV4z2VcH2TA0/edit?usp=sharing).

<https://docs.google.com/document/d/1WvdeC1atp7J-ZKWOUkSLsEcosjZ0AqV4z2VcH2TA0/edit?usp=sharing>



# Objectives:

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## 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 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.

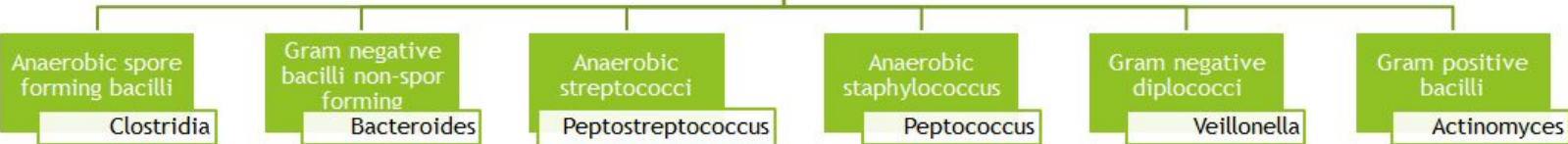
# Doctor's Notes:

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- ★ Anaerobic bacteria do not need oxygen to grow, and they can cause a different type of infections.
  - Usually, the infection would be around the areas that we have Anaerobic bacteria as normal flora (oral cavity, upper respiratory, GIT).
  - Most of the anaerobic bacteria lack the enzymes (Catalase & Peroxidase).
  - Some of them lack the enzyme (superoxide dismutase).
  - They are not easy to culture in the laboratory.
  - and it's very sensitive to oxygen.
  - Anaerobic bacteria are slow to grow (2-5 days).
- ★ In laboratory diagnosis, why we culture anaerobic bacteria in liquid media like cooked meat?
  - Because it contains nutrient & proteins, which can help the anaerobic bacteria to grow.
- ★ The most important anaerobic bacteria is *Bacteroides fragilis* (B.Fragilis) because it's the most common and the most resistance bacteria.
  - B. Fragilis group are normal flora in GIT, (below the waist, non-pigmented, and more common\*^.)
- ★ Actinomyces; is the normal flora in the oral cavity.
- ★ Gram +ve bacilli spores forming are the most important group, because it's only one genus (Clostridium genus).
  - But they have different species and each species can cause a different disease.
- ★ *Cl. perfringens* is normal flora in GIT of ( human and animals ), and they can cause Gas gangrene\*.
  - \*Gas gangrene: is a type of wet gangrene caused by *Cl. perfringens*, which leads to necrosis.
  - *Cl. Perfringens* : ( large Gram +ve bacilli with Few or No WBCs ) under the microscope,
  - they produce lecithinase, that kills the WBCs.
- ★ *Cl. Tetani* is normal flora in all animals, mainly horses, and we found it in soil التربة .
  - Causes: contraction.
- ★ *Cl. Botulinum*, we found it in the soil.
- ★ *Cl. Difficile*: normal flora in human (GIT),
  - This organism we found it a lot in hospitals because they are resistant to alcohol "they love alcohol".
  - And they can be transmitted.
  - The diagnosis by stool culture.

- Anaerobes: are bacteria that would die if exposed to a very minimal amount of oxygen. (also called obligatory or strict anaerobes).
- Facultative Anaerobes (F.A.): can live as aerobics and anaerobes (makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent ). Most of the well known clinically significant are F.A.
- aerotolerant: are anaerobes that need a very minor amount of oxygen to grow.

## CLASSIFICATION

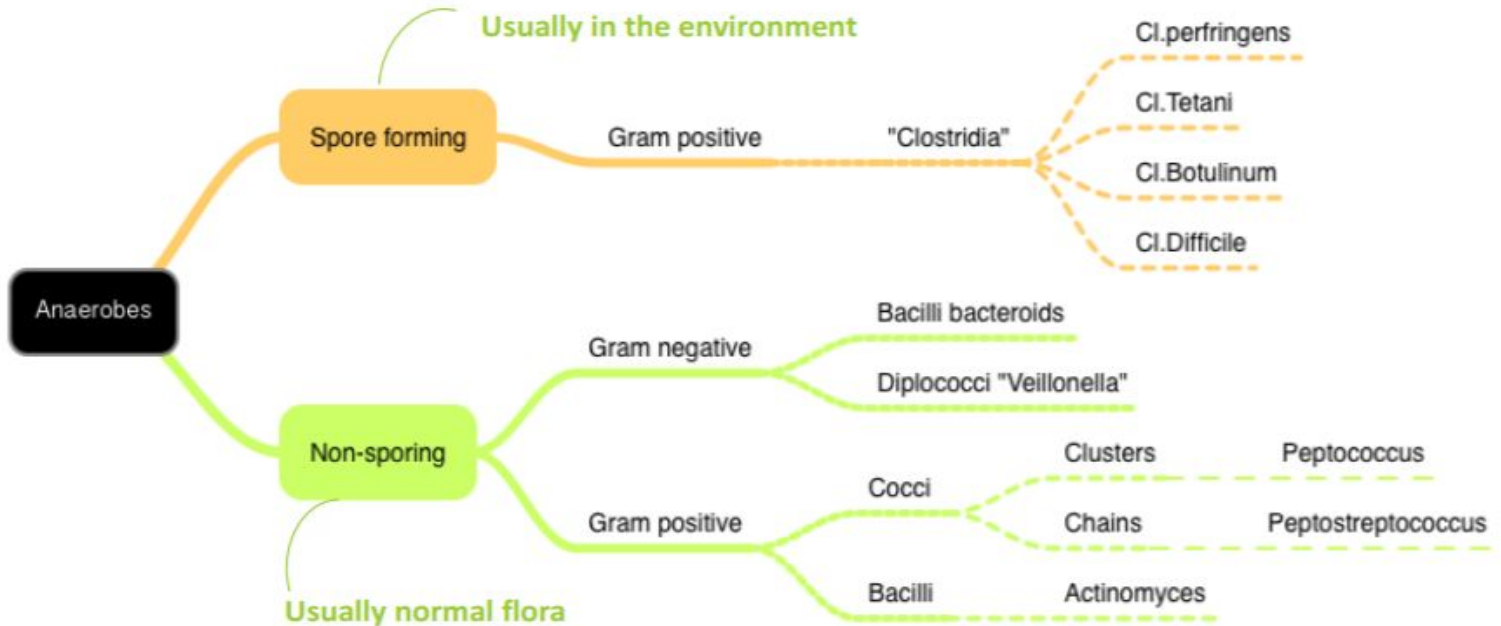


Are usually found in soil

## ANAEROBIOSIS

- ▶ Lack cytochrome → cannot use oxygen as hydrogen acceptor( Most Lack Catalase & Peroxidase)
- ▶ Contain flavoprotein so in the presence of oxygen produce H<sub>2</sub>O<sub>2</sub> 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

# Classification of anaerobes:



# Habitat I :

## Oropharynx ( mouth ):

- e.g.
- Fusobacteria
- Prevotella melaninogenica
- Veillonella

## Gastrointestinal tract

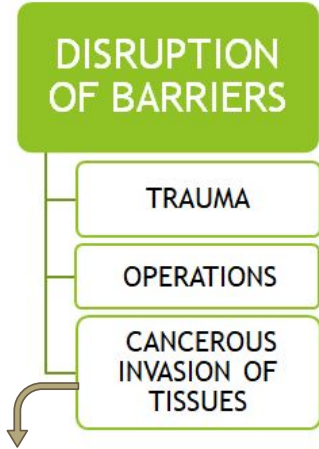
- Found mainly in the large colon in large numbers
- Total number of anaerobes =  $10^{11}$
- While all aerobes (including E. coli) =  $10^{14}$
- examples are ( 1 ) Bacteroides fragilis ( 2 ) Bifidobacterium specie

## Female genital tract (mainly in the vagina)

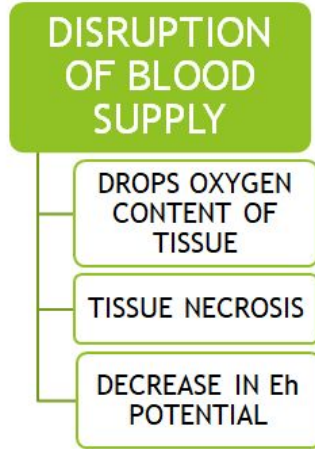
# FEATURES OF ANAEROBIC INFECTIONS

1. Infection from animal bites. Also human bite
2. Deep abscesses
3. The infections are also polymicrobial
4. Gas formation, foul smell رائحة كريهة
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 ?



Especially colon bacteria



Most famous example is diabetic patients

## WHAT ARE THE INFECTIONS CAUSED BY THESE ANAEROBIC ORGANISMS ?

- 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

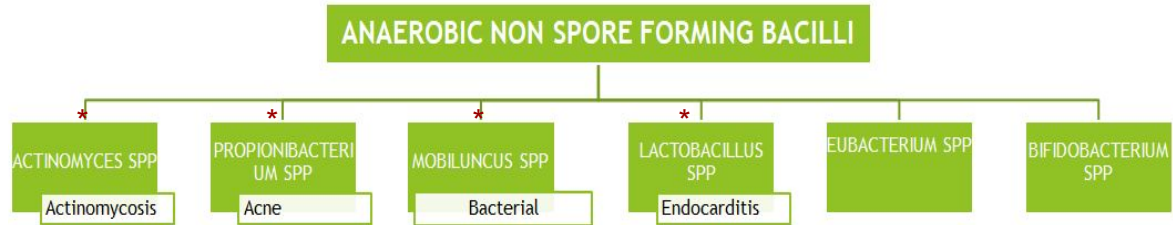
# LABORATORY DIAGNOSIS:

## When anaerobic infection is suspected:

- Specimens have to be collected from the site containing necrotic tissue.
- Pus is better than swabs. *العينات توضع في وعاء مغلق*
- Specimens has to be send to the laboratory within 1/2 hour why? they will convert O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub>(toxic). then, they will die. See slide 3
- 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.
- But penicillin can be used for other anaerobes .
- Flagyl (metronidazole) is the drug of choice.
- Clindamycin can also be used.





# Anaerobic bacteria

Note : the words which are highlighted in red are what the doctor focused on, and it details in next slides



## Non spore forming

### Gram positive

### Gram negative

#### Bacilli

#### cocci

#### Bacilli

#### cocci

- **Actinomyces** spp causing : actinomycosis
- Propionibacterium spp causing : acne
- Mobiluncus spp causing: bacterial vaginosis
- Lacto bacillus spp causing :endocarditis
- Eubacterium spp
- Bifidobacterium spp

- Peptococcus  
In cluster
- **Peptostreptococcus**  
In chains causing : **Brain abscess**

- **Bacteroides**
- **Fusobacterium**
- Prevotella
- Porphyromonas
- succinomonas
- Butyrivibrio

- Veillonella (diplocci)  
The only one

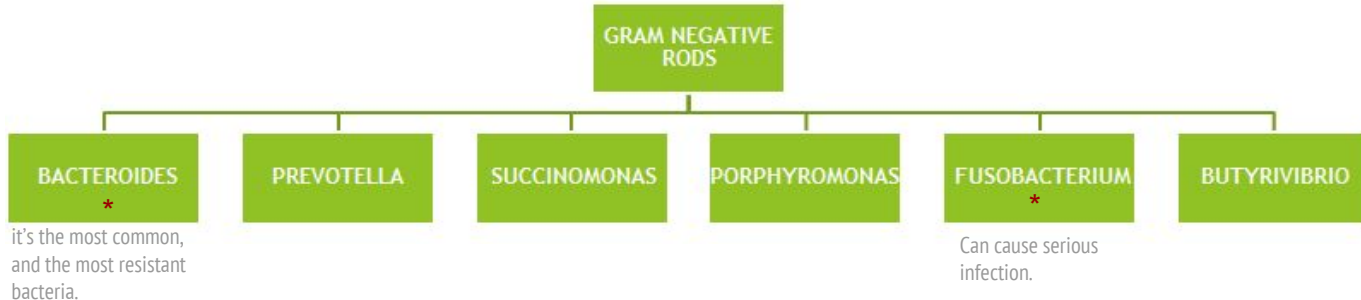
# ACTINOMYCOSIS

case scenario اغلب أسئلتها

\*IUD = intrauterine device.

- 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



## •STRICT ANAEROBE PLEOMORPHIC (COCCO BACILLI)GRAM NEGATIVE BACILLI

<b>Bacteroides fragilis group</b>	<b>Bacteroides species other than b. Fragilis group</b>
Account for 1/3 of all isolates Normal flora in Git trac. ( it's below the waist, non-pigmented, and more common. )	Less common flora in Oral cavity.
B. FRAGILIS, B. VULGARIS, B.THETA IOTAMICRON B. UNIFORMIS	Prevotella Porphyromonas
Resistant to 20% bile	Bile sensitive
Resistant to many antibiotics Penicillin, kanamycin, vancomycin, colistin – and many more	Resistant to kanamycin only
•No pigmentation of colonies or fluorescence (more resistant and more common to cause infection.)	Some pigmented

<p><b>OTHER ANAEROBIC GRAM NEGATIVE BACILLI</b></p>	<p><b>ANAEROBIC GRAM POSITIVE COCCI</b></p>		<p><b>ANAEROBIC GRAM NEGATIVE COCCI</b></p>
<p>BACILLI</p>	<p>CHAINS</p>	<p>CLUSTERS</p>	<p>SHEET-LIKE</p>
<p><b><u>FUSOBACTERIUM NECROPHORUM</u></b></p>	<p><b><u>PEPTOSTREPTOCOCUS</u></b>          لما تكون موجودة = Brain abscess.</p>	<p><b><u>PEPTOCOCCUS</u></b></p>	<p><b><u>VEILLONELLA PARVULA</u></b></p>
<p>PERITONISILLAR →          INTRNAL JUGULAR VEIN          THROMBOSIS → EMBOLI          TO THE LUNG LEMIERRE          SYNDROM</p>	<p><b>BRAIN ABSCESS,</b></p>	<p>-</p>	<p>-</p>

# LARGE GRAM POSITIVE SPORE FORMATION BACILLI : CLOSTRIDIUM SPECIES

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CLOSTRIDIUM SPECIES	MAJOR DISEASE
<i>Cl. perfringens</i> and other e.g septicum	Gas gangrene
<i>Cl. tetani</i>	Tetanus
<i>Cl. botulinum</i>	Botulism
<i>Cl. difficile</i>	4. Toxic enterocolitis (Pseudomembranous colitis)

Cl = CLOSTRIDIUM

# Clostridium Species: (spore forming, gram positive bacilli)

Bacteria	Origin:	Pathogenesis:	Disease:	laboratory Diagnosis:	Treatment and prevention:
<p><u><i>Clostridium perfringens</i></u> <u>(<i>Cl. welchii</i>)</u></p> <p>Morphology:</p> <p>large rod Gram +ve with bulging sub-terminal endospores</p>	<p>Normal flora of GIT (human &amp; Animals* )</p> <p>Spores contaminate From soil wound</p>	<p>Traumatic open wounds or compound fractures lead to muscle damages and contamination with dirt etc,</p> <p>•Mainly in war wounds, old age, low blood supply and amputation of thigh (required prophylaxis with penicillin)</p> <p>بروفيلاكسس = علاج للوقاية (من مرض)</p>	<p>1) Wound infection after wound and Contamination can lead to Gas Gangrene - most serious disease</p> <p>•2) Gas Gangrene of the uterus after criminal abortion</p> <p>•3) Food Poisoning : Spores are swallowed → germinate in gut after 18 hours (Toxin production) → abdominal pain and diarrhoea</p>	<p>Smear Gram stain Large Gram positive bacilli <b>with few or no WBCs.</b></p> <p>•Culture</p> <p>•Blood agar with haemolytic colonies (double zone of haemolysis )</p> <p>•Cooked meat medium</p> <p>•Gives the NAGLAR'S Reaction &amp; toxin neutralization on Egg yolk medium &amp; toxin is a phospholipase</p> <p>(اختبار ناقلار يفرق بين البيرفرنجنس و غيرها من الكلوستريديم وهو يستعمل فيه صفار البيض cooked meat medium)436</p>	<p>•Remove dead tissue , debris and foreign bodies .Penicillin and hyperbaric oxygen (اعطاء المريض اوكسجين نقي و مضغوط in some cases</p>

# CONT.



[for better understanding](#)

Bacteria	Origin:	Pathogenesis:	Disease / clinical features:	laboratory Diagnosis:	Treatment and prevention:
<p><u><i>Clostridium .tetani</i></u> (<b>Tetanus</b>)</p> <p><b>terminal spore</b> (called drumStick appearance)</p> <p>gram positive</p>	<p>Lives in soil and animal feces. e.g horse and any wound can infected if contaminated by spores</p> <p>Normal flora in all animals, mainly horses.</p>	<ul style="list-style-type: none"><li>•Mainly due to <b>tetanospasmin</b> which is powerful exotoxin (protein) .</li><li>•This organism does not lead to invasion or Bacteraemia . Its function to <b>inhibits transmission of normal inhibitory messages</b> from <b>CNS</b> at anterior horn cells of cord.</li></ul>	<ul style="list-style-type: none"><li>•Incubation period 1-3 weeks (time from infection to the appearance of symptoms)</li><li>•Face &amp; neck <b>wounds</b> are more <b>dangerous</b>.</li><li>•Symptoms: local (not common), cephalic (rare), generalized (<b>most</b> common)</li><li>•Painful muscle spasm around infected wound and Contraction of muscles in the face called <b>Trismus</b> (Lockjaw) , <b>Risus Sardonicus</b> (<b>facial muscle</b>)</li><li>•<b>Opisthotonus</b> التشنج الظهري (<b>Araching of Back</b>)in children.</li><li>•<b>Opistho</b> meaning "behind" and <b>tonos</b> meaning "tension", due to extrapyramidal effect and is caused by spasm of the axial along the spinal column .</li></ul>	<p>Mainly by clinical and it is strict anaerobe very motile , spread on agar.</p>	<p><b><u>Treatment</u></b></p> <ul style="list-style-type: none"><li>•Cleaning of wound and removal of Foreign body</li><li>•Specific by antitoxin form horse serum but it can lead to anaphylaxis &amp; shock must be tested first or human immunoglobulin.</li><li>•Antibiotics : <b>Penicillin</b>.</li><li>•Supportive treatment by keeping the patient in dark pace, fluids and sedative valium</li></ul> <p><b><u>Prevention</u></b></p> <ul style="list-style-type: none"><li>• by vaccination</li></ul>

# CONT.

## Botulism (التسمم من هذه البكتيريا)

- 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

Bacteria	Origin:	Pathogenesis:	Disease / clinical features:	laboratory Diagnosis:	Treatment and prevention:
<p><u><i>Clostridium botulinum</i></u></p> <p>(Toxin is exotoxin (protein) heat labile at 100 °C and resist gastrointestinal enzymes)</p>	<ul style="list-style-type: none"> <li>• Found in soil ponds ,, lakes, canned food, sea food (salmon) and honey.</li> </ul>	<ul style="list-style-type: none"> <li>• Attacks neuromuscular junctions and prevents release of <b>acetylcholine</b> that can leads to paralysis</li> </ul>	<ul style="list-style-type: none"> <li>• Abnormal eye movement as if cranial nerve affected when bulbar area of the brain affected.</li> <li>• Finally the patient might develop respiratory and circulatory collapse</li> <li>• <u>Infantile Botulism</u> Ingestion of <b>Spores</b> → <i>germination in the gut</i> → <b>Botulism</b> .<b>Child present with week</b> child, cranial nerve and constipation</li> </ul>	<ul style="list-style-type: none"> <li>• Suspected food from the patient <b>faeces culture</b> or serum toxin detection by mice inoculation after weeks → <b>paralysis and death</b></li> </ul>	<p><u>Treatment</u></p> <ul style="list-style-type: none"> <li>• Mainly supportive and horse antitoxin in sever cases +penicillin</li> </ul> <p><u>Prevention</u></p> <ul style="list-style-type: none"> <li>• Adequate pressure cooking autoclaving and heating of food for 10 minutes at 100 °C</li> </ul>

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



# CONT.

Bacteria	Origin:	Pathogenesis:	Disease / clinical features:	laboratory Diagnosis:	Treatment and prevention:
<u><i>Clostridium Difficile</i></u>	Normal flora in GIT	<ul style="list-style-type: none"><li>• after exposure to antibiotics and killing of other normal flora, <b>this organism will multiply</b> with then produce toxin that has two components</li><li>• <b>toxin (A)–Subunit enterotoxin</b> (cause diarrhea)</li><li>• <b>toxin (B)-Subunit Cytotoxic</b> ( kill the cells ie necrosis)</li></ul>	<p><b>PSEUDOMEMBRANE COLITIS</b> is the clinical manifestation of this disease which composed of bacteria , fibrin , WBCs and dead tissue cells</p> <p><b>Severe dehydration</b> , intestinal obstruction and perforation are some of complication of this syndrome</p>	This organism hard to grow in the laboratory required special media and growth of the organism in <b>solid media required cell line culture</b> to illustrate cytotoxicity of the organism. <b>The simplest method for diagnosis by detection of the toxin in the stool by immunological testing (ELISA)</b>	<p><b>Treatment :</b></p> <p><b>Metronidazole</b>_or and oral vancomycin in severe cases</p> <p><b>Prevention:</b></p> <p><b>This organism form spores and hard to control in the hospital because they are resistant to alcohol</b> decontamination (<u>use Na hypochloride instead</u>).</p> <p>•Patient need to be <b>isolated</b> and contact need to be <b>screened</b> to find out if they carrying the toxic strain of the bacteria.</p>

# Notes on anaerobic bacteria

Anaerobic bacteria is similar to aerobic bacteria that they both have Gram positive bacilli, Gram negative bacilli, Gram positive cocci.. But the major one is **Clostridium** (which is anaerobic, spore forming Gram positive bacilli ).

**Gram positive bacilli: can be both aerobic or anaerobic.**

Gram negative bacteria **Cannot form spores.**

Clostridium is like viruses in a way that **one Clostridium can cause infection** by itself (can cause **different clinical presentations by itself**)

Any single species of Clostridium can cause totally different clinical presentations

We have 4 major species:

- 1) Clostridium tetani ( cause spasm )
  - 2) Clostridium perfringens ( cause gas gangrene ) , release a toxin called Phospholipase
  - 3) Clostridium botulinum ( cause paralysis )
  - 4) Clostridium *difficile* ( cause diarrhea )
- \* التيتاني والبوتولينيم عكس بعض بحيث الاول يسبب انقباض للعضلات اما الثاني يسبب شلل

## Notes from MicroTeam 436

Clostridium *difficile* ( cause diarrhea )

هذي تفرز نوعين من التوكسينز

A enterotoxin which causes diarrhea

B cytotoxic ( kill the cells )

Clostridium are commonly found in soil and are able to survive under adverse conditions

It is common in any infection that their will be an increase in WBC number, but in clostridium perfringens ( that causes gas gangrene ) we will have low WBC!! Why?

Because they produce leukos...( toxin that kill WBC )

# MCOs:

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**1. Treatment of most anaerobic bacteria is:**

- a) Penicillin      b) Metronidazole      c) Vancomycin

**2. Anaerobic bacteria lack an enzyme called:**

- a) Lactase      b) Superoxide dismutase      c) Lyase

**3..... is the most location in anaerobic bacteria:**

- a) Genital tract      b) GIT      c) Respiratory Tract

**4. The broad classification of bacteria is based on the types of reactions they employ to generate energy for growth?**

- a) T      b) F

**5. Is resistant to penicillin?**

- a) Clostridium      b) fusobacterium      c) Bacteroides fragilis

**6. - What causes toxic enterocolitis?**

- a) cl. Difficile      b) cl. tetani      c) cl. botulinum

# Team Leaders:

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## Members:

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بدر القرني ★

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- عبدالله الداود ★
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