MED437
KING SAUD UNIVERSITY

MICROBIOLOGY
437

Microbiology – Lecture Anaerobes of medical importance

TEAM 437

Red: important
Green : doctor notes
Black : original slides
Grey: extra information

In this link, you will find any corrections or notes unmentioned in the team's work. Please check the link below frequently.

https://docs.google.com/presentation/d/1yIQt3G8UDFG6xYMRhXkTk-dS54NeTfhJaPe_y0M-kjk/edit?usp=sharing

Objectives

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- 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.
- 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.

Anaerobes

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Anaerobiosis: Microbes that can only grow under anaerobic conditions. They lack cytochrome so they cannot use oxygen as a hydrogen acceptor. Most Lack Catalase & Peroxidase.

Contain flavoprotein so in the presence of air that has >10% oxygen they produce H2O2 which is toxic. They are also sensitive to metronidazole (MTZ).

Some lack an enzyme called superoxide dismutase so many killed ,peroxide and toxic radicles enzyme like fumarate reductase must be in reduced form to work

Why can't anaerobic bacteria survive in oxygen?
The presence of oxygen leads to the production of the superoxide radical (a negatively charged O2 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. Peroxide and toxic radicals enzymes like fumarate reductase must be in their reduced form to work.

These organism are normal flora in the:

A. Oropharynx (the base of the tongue, the tonsils, the soft palate, and the walls of the pharynx)
eg. Provetella melaninogenicus, Fusobacteria, Veillonella.

B. Gastrointestinal tract

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

C. Female genital tract (mainly in the vagina).

D. Skin

- Differentiate the various types of anaerobes with regard to atmospheric requirement (i.e. obligate anaerobes, Facultative anaerobes and aerotolerant anaerobes).

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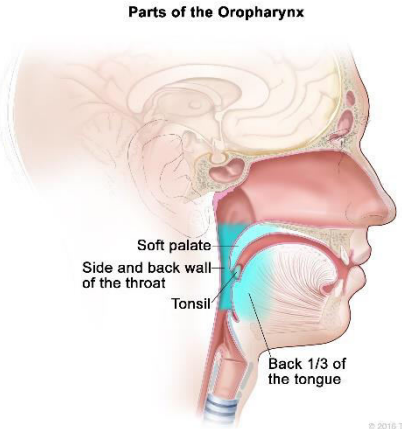
TABLE 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Features of anaerobic infections:



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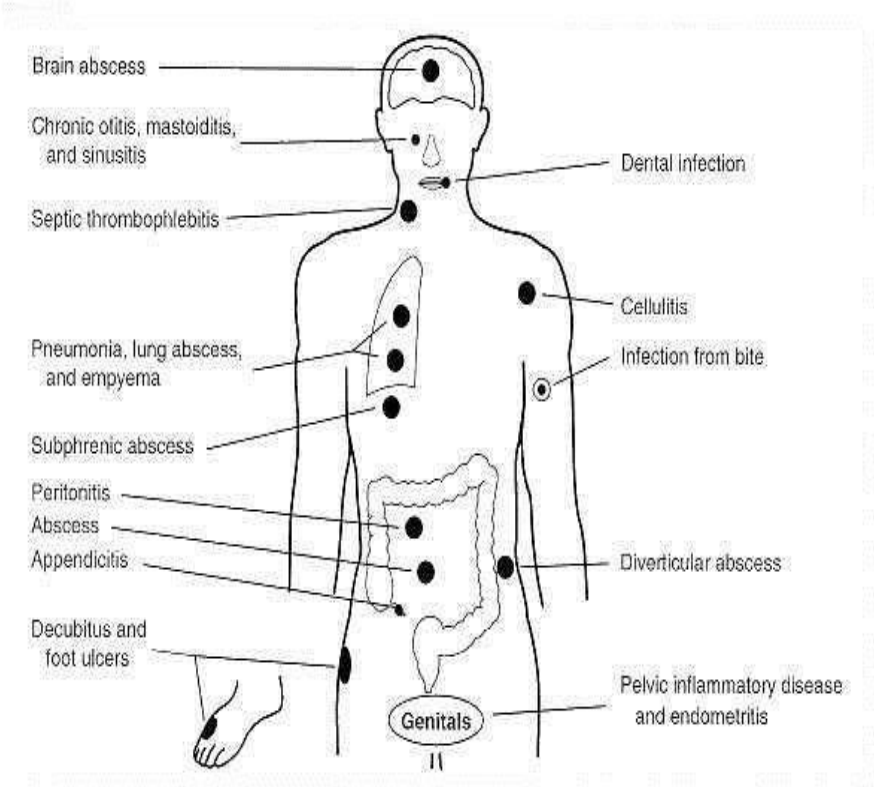
(Just read through it)

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.



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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

Continue

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 perfreingens (that causes gas gangrene) we will have low WBC!! Why?

Because they produce leukos…(toxin that kill WBC)

Classification of anaerobes:

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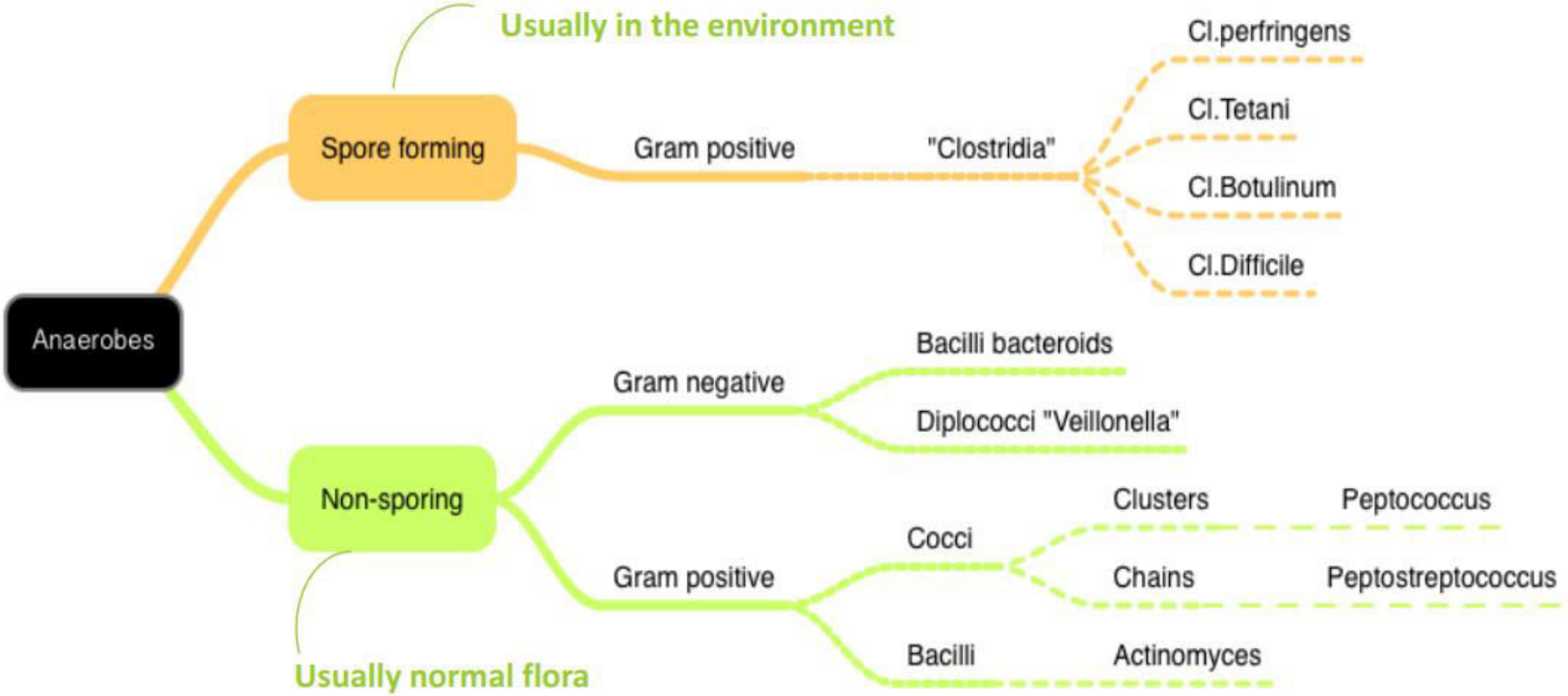

For more info

Infection

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How does the infection begin?

Disruption of barriers
- Trauma
- Operations
- Cancerous invasion of tissue

Disruption of blood supply
- Drops oxygen content of tissue
- Decrease in Eh potential
- Tissue necrosis

Infections caused by anaerobic organisms:	**Almost all infections are indigenous except:**
- **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.	○ Tetanus ○ Infant, wound botulism ○ Gas gangrene (some cases) ○ Bites ○ C .difficile (nosocomial)

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Laboratory Diagnosis:

when an anaerobic infection is suspected;

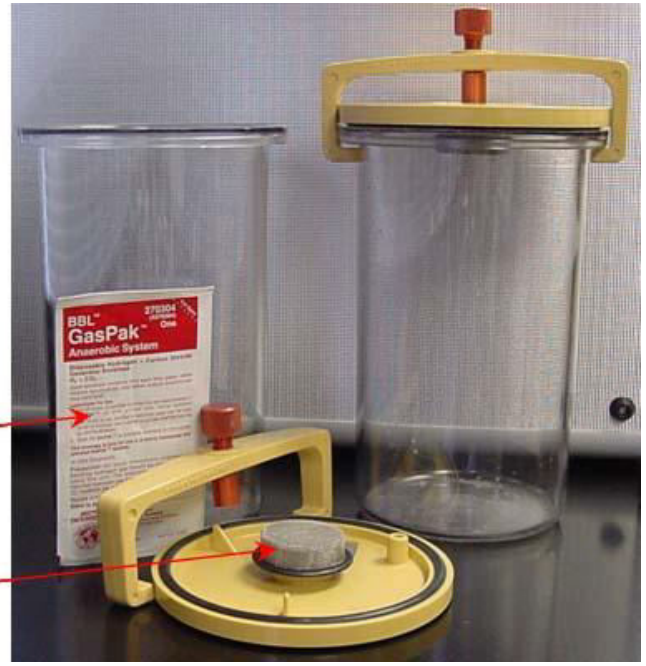
a) **Specimens** have to be collected from the site containing necrotic tissue.

b) **Pus is better** than swabs.

c) Specimens have to be **sent** to the laboratory **within 1/2 hour why?** otherwise the bacteria dies.

d**) Fluid media like cooked meat broth** are the best culture media because they provide nutrition in an anaerobic environment.

e) Specimens are preferably extracted from abscesses or deep wounds and **incubated anaerobically** (in nitrogen) for 48 hours.



GasPak Envelope

Wire mesh containing palladium catalyst

Treatment

- Penicillin can be used for many anaerobes
- Bacteroides fragilisis always resistant to penicillin.
- But penicillin can be used for other anaerobes
- Amoxacillin clavulanic acid in case of beta-lactamases producer
- Flagyl(metronidazole) is the drug of choice.
- Clindamycincan also be used.
- Vancomycin can be use only for clostridium difficile
- Anaerobes all resistant to aminoglycosides
- Pipracillin and carbapenems in sever infection

Character of anaerobic infection

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1. Suppuration
2. Abscess formation
3. Tissue destruction (gangrene)
4. Septic thrombophlebitis

ONLY FEMALES SLIDE

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

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Anaerobic bacteria

Non spore forming

Note : the words witch are highlighted in will be explained in red are what the doctor focused on, and it details in next slides

Gram positive

Bacilli

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

cocci

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

Gram negative

Bacilli

- **Bacteroides**
- **Fusobacterium**
- Prevotella
- Porphyromonas
- succinomonas
- Butyrivbrio

cocci

- Veillonella (diploocci)
  The only one

Most important bacteria

Clostridium species: (spore forming, gram positive bacilli)

الجدول محدد على الي نبه عليه الدكتور

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Bacteria:	Origin:	Pathogenesis:	Disease :	Diagnosis:	Treatment and prevention:
Clostridium perfringens (Terminal)	GIT (human) Spores contaminate From soil→ wound	-Traumatic open wounds or compound fractures lead to muscle damages and contamination with dirt -Mainly in war wounds, old age, low blood supply and amputation of thigh phospholipidesases	1) Wound infection 2) Gas Gangrene - most important disease 3) Food Poisoning : Spores are swallowed → Germinate in gut after 18 hours(Toxin production)→ abdominal pain and diarrhea	-hemolysis -Toxin detection	penicillin
Clostridium Tetani (Subterminal) Should be terminal	GIT (animal) Spores contaminate From soil→ war, birth	Tetanospasmin block inhibitory message from CNS	Painful muscle spasm around infected wound can be local or generalized	Mainly by clinical and it is strict anaerobe very motile , spread on agar.	Penicillin Antitoxin vaccine
Clostridium Botulinum (terminal)	-Caned food, honey and sea food stored in soil - Enviroment	Powerful exotoxin	- Muscle paralysis - Infantile boutulism (تسمم حديثي الولادة) by ingestion of spores	culture	Penicillin Antotoxine vaccine
Clostridium *Difficile* (terminal) Can be transmitted from person to person	GIT (human) Transmit in hospital to other patients	Toxin A,B (enterotoxin and cytoxin)	-diarrhea -Toxic megacolon -Pseudomembranous colitis	Culture Toxin detection by: ELISA PCR and cell line	-Metronidazole -Vancomycin (oral) -Stool transplant

Large gram + Bacilli Spore-forming	Origin	Pathogenesis	Diseases	Diagnosis	Treatment and prevention
C.Perfringens *Subterminal*	GIT (human) Spores contaminate Farm soil→ wound	Phospholipases	Gas gangrene Necrotizing Fasciitis Food poisoning	Boxcar bacilli Double zone hemolysis Toxin detection	Penicillin
C.tetani *Terminal*	GIT (animal) Spores contaminate Farm soil→ war, birth	Tetanospasmin Block inhibitory message from CNS at anterior horn	Muscle spasm Local, cephalic (Jaws) Generalized (back)	Terminal spores Culture Spread on agar (motile)	Penicillin Antitoxin Vaccine
C.Botulinum *Subterminal*	soil ponds and lakes Caned food, honey and sea food stored in soil	Powerful exotoxin prevents release of acetylcholine at neuromuscular junctions	Muscle paralysis Infantile botulism: ingestion of spores in food	Culture Animal inoculation	Penicillin Antitoxin Vaccine
C. Difficile *Subterminal*	GIT (human) Transmit in hospital fo other patients Due over use of antibiotics	Toxin A,B (enterotoxin and cytoxin)	Antibiotics associated diarrhea Toxic megacolon Pseudomembranous colitis	Culture Toxin detection by PCR ELISA Cell line	Metronidazole Vancomycin (oral) Stool transplant

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بدون تحديد

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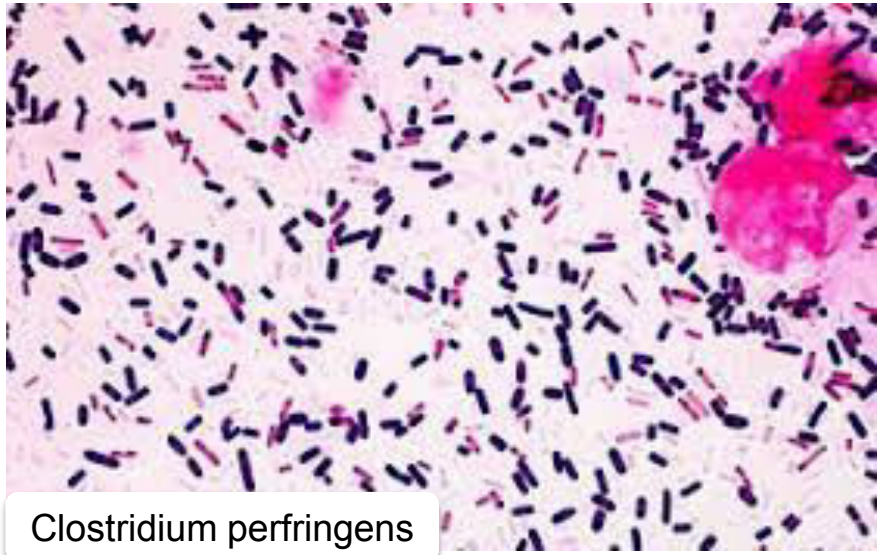


Clostridium perfringens

Clostridium tetani

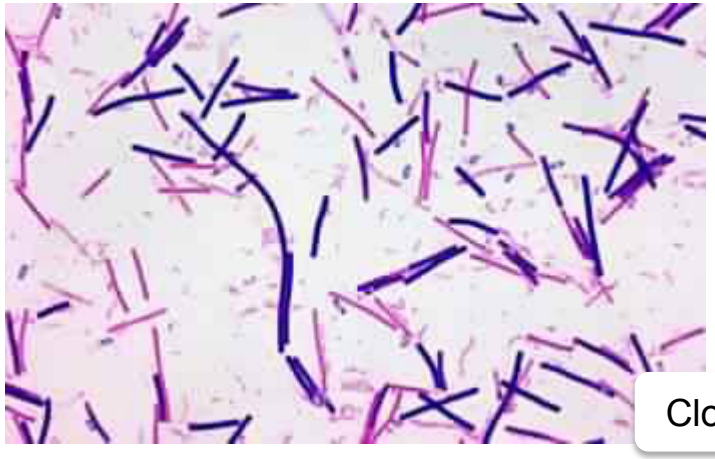
NOTE:

- Dominate the indigenous flora (colonization resistance)
- Commonly found in infection
- Easy to overlook(Special precautions)

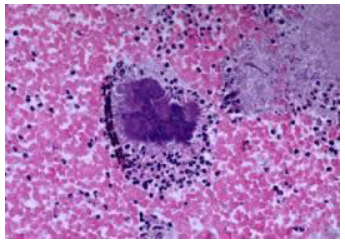
a) Slow growth
b) Mixed infection

- Difficult treatment

Clostridium Botulinum



Clostridium difficile



Most important bacteria cont.

Non- Spore Forming Bacilli :

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1- Actinomycosis Gram negative	2-Propionibacterium speices: Gram positive	3- Lactobacillus:
•branching shape popcornshaped anaerobic “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.	•Causes acnes (حب الشباب) •living in and around the sweat glands, sebaceous glands, and other areas of the skin. •To prevent the incidence of scarring, never squeeze or pick acne lesions and cleanse the skin using a soft warm cloth 	•used to treat lactose intolerance 

Most important bacteria Gram negative:

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It has a fusiform morphology
Not resistant or common
Can KILL young people

1- Fusobacterium

Gram negative

Peritonsillar leads to internal jugular vein.

Thrombosis leads to emboli to the lung.

2-Bacteriodes

- Account for 1/3 of all isolates
- Resistant to many antibiotics (Penicillin, kanamycin, vancomycin, colistin)
- Resistant to 20% bile
- No pigmentation of colonies or fluorescence
- Metronidazole is used for treatment
- Has 4 species: B. Fragilis (only resistant to kanamycin) , B.Vulgaris , B.Thetaiotamicron , B.Uniformis

They are also:

- Strict anaerobe
- Pleomorphic
- Gram negative bacilli (cocco bacilli)
- Normal flora in
- Oropharynx
- Gastrointestinal tract
- Vagina

Bacteroides species **<u>other</u>** than b. Fragilis group are :

- Bile sensitive
- Resistant to kanamycin only
- Some pigmented

Cocci shaped anaerobes

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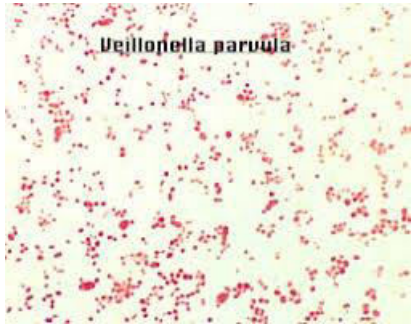

Gram positive:	Gram negative:
1. Peptococci (staphylococcus) ➢ Found in mouth and GIT ➢ Characterized by causing wounds ➢ Can be cultured by using a smear culture ➢ Is treated using Clindamycin 2- Peptostreptococci (streptococci) ➢ Found in mouth and GIT ➢ Characterized by causing brain abscess ➢ Can be cultured by using a smear culture ➢ Is treated using Clindamycin	✓ Viellonella species. (diplococci) ➢ Found in mouth and GIT ➢ Characterized by causing wound abscess ➢ Can be cultured by using a smear culture ➢ Is treated using Metronidazole ONLY GRAM NEGATIVE ANAEROBIC COCCI

Questions

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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 for anaerobic infection.

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) Closteridium b) fusibacterium c) Bacteroides fragilisis

6. - What cuses toxic enterocolitis?

a) cl.Diffcle b)cl.tetani c) cl.botulinuim

1.A 2.B 3.B 4.A 5.C 6.A

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