Microbiology
team 436

436
MEDICINE
KING SAUD UNIVERSITY

microbiology

LECTURE: ANAEROBIC

IMPORTANT.
DOCTORS NOTES.
EXTRA INFORMATION.

ملاحظة مهمة جداً:
هذه المحاضرة معتمدة على سلايدز الدكتور لذا يوجد شرائح من محاضرة الدكتورة غير موجودة.

Objectives

Describe anaerobic bacteria including their sensitivity to oxygen and where they may be found in the environment and the human body.

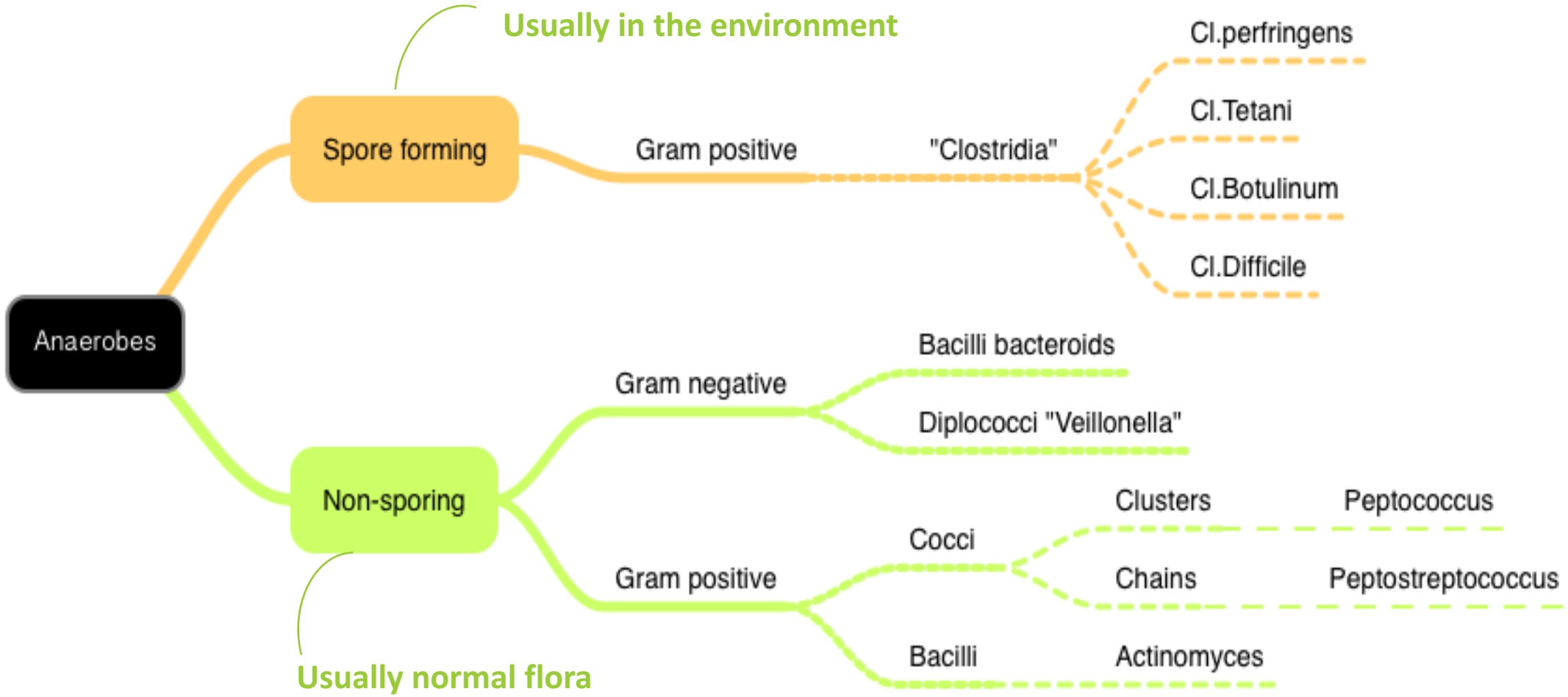
- Differentiate the various types of anaerobes with regard to atmospheric requirement (i.e. obligate anaerobes, Faculative anaerobes and aerotolerent anaerobes.
- Describe how anaerobes, as part of endogenous microbiota, initiate and establish infection.
- Name the endogenous anaerobes commonly involved in human infection.
- Recognize specimens that are acceptable and unacceptable for anaerobic culture.
- 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.

objectives

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 anaerobics:



Anaerobiosis

Lack cytochrome so they cannot use oxygen as hydrogen acceptor.

Most Lack Catalase & Peroxidase (Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen)

Contain flavoprotein so in the presence of oxygen produce H2O2 which is toxic

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

1) Catalyzes Superoxide O_2^- radical into oxygen and H_2O_2
2) Its an Anti-oxidative

Superoxide O2- are similar to free radical

- Highly toxic
- Highly reactive

DEFENITION:

-A MICRBE THAT CAN ONLY GROW UNDER ANAROBIC CONDITION
SENSETIVE TO metronidazole (MTZ)
FAIL TO GROW IN AIR 10 % O_2

This box is Only in female's slides

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)

HABITAT I :

These organism are normal flora in:

A. **Oropharynx** *eg. 1. Provetella melaninogenicus*
   2.Fusobacteria
   3. Veillonella

B. **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*

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

FEATURES OF ANAEROBIC INFECTIONS:

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.

HOW DOES THE INFECTION BEGIN ?

-DISRUPTION OF BARRIERS

TRAUMA
OPERATIONS
CANCEROUS INVASION OF TISSUES

-**DISRUPTION OF BLOOD SUPPLY**

DROPS OXYGEN CONTENT OF TISSUE
DECREASE IN Eh POTENTIAL
TISSUE NECROSIS

WHAT ARE THE INFECTION CAUSED BY THESE ANAEROBIC ORGANISMS :

-Post operative wound infection

-Brain, dental, lung abscess

-Intra abdominal abscess, appendicitis, diverculitis

-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;

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** why?

d) **Fluid media like cooked meat broth** are the best culture media.

e) Specimens have to **incubated anaerobically** for 48 hours. **Because they are slowly growing pathogens**

TREATMENT:

-*Bacteroides fragilis* is always resistant to penicillin.
But penicillin can he used for other anaerobes

-Flagyl (metronidazole) is the drug of choice.

-Clindamycin can also be used.

CHARACTER OF ANAEROBIC INFECTION:

-Suppuration
-Abscess formation
-Tissue destruction{gangrene}
-Septic thrombophlebitis
-Some have unique pathology :
Actinomycosis
Psedomembranous colitis
Gas gangrene

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

Only in female's slides

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

E.g. of non spore forming anaerobic gram (+) bacilli

ACTINOMYCOSIS

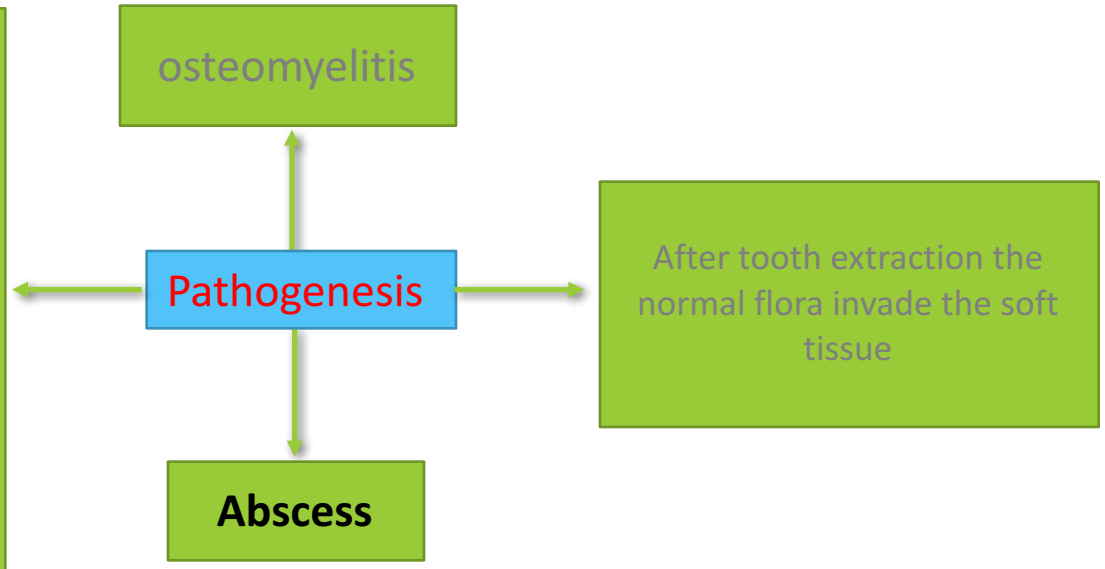
Definition : **branching beaded anaerobic** "microaerophilic" gram positive bacilli

-It is a **normal flora** in : oral cavity, GIT, genital track

Actinomycosis:
Source of infection is normal flora in normal host cell

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

Can spread to the brain, liver, bone and blood



Diagnosis : gram stain **with sulfur granules** and growth of **molar tooth colonies**

Treatment : **penicillin** ,tetracycline or clindamycin

E.g. of non spore forming anaerobic gram (-) bacilli

fusobacterium

It has a fusiform morphology

Normal flora in : oral cavity, GIT

Fusobacterium necrophorum

What does it cause?
Peritonsillar leads to Internal jugular vein. Thrombosis leading to emboli to the lung

Treatment : **penicillin**

Bacteroides

It is a pleomorphic bacteria (cucco bacilli)
Strict anaerobe : the **most resistant anaerobic** bacteria
the **most anaerobic bacteria** causing infection

Normal flora in : GIT, vagina, oropharynx

	B.fragilis	Other than B.fragilis
Examples	Only B.fragilis	B.Vulgaris B.Thetaiotamicron B.Uniformis
Properties	-account for 1/3 of all isolates -**resistant to 20% bile** And many antibiotics such as : **penicillin**, kanamycin, vancomycin, colistin -**no pigmentation** of colonies or fluorescence	-bile sensitive -resistant to kanamycin only -some pigmented

Treatment : **metronidazole** (flagyl)

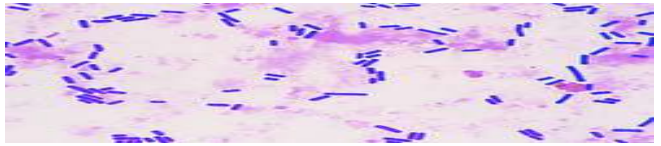
Clostridium Species

-Morphology: Large gram positive rods. **-Spore forming.** **-Causative agents (مسببة) 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)



-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.

اختبار ناقلار يفرق بين البيرفرنجنس و غيرها من الكلوستريديم وهو يستعمل فيه صفار البيض cooked meat medium

Continued…

Can leads to the following diseases:

1) Wound Contamination
2) Wound infection
3) Gas Gangrene - most important disease
4) Gas Gangrene of the uterus in criminal abortion (يقصد بالاجهاض الغير قانوني انه لا ينفذ بالطريقة الصحيحة او بالادوات المناسبة احيانا)
5) Food Poisoning : Spores are swallowed → Germinate (تتكاثر) in gut after 18 hours(Toxin production)→ abdominal pain and diarrhea

-Pathogenesis: Traumatic open wounds or compound fractures (كسر يخترق الجلد و يسبب انفيكشن) 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). (بروفيلاكسيس يعني علاج او اكشن نتخذه في سبيل الوقاية من مرض عادة مرض محدد بذاته)

Caused by the bacteria's alpha toxin called phospholipase C.

Prevention and Treatment: Remove dead tissue , debris and foreign bodies .Penicillin and hyperbaric oxygen (اعطاء المريض اوكسجين نقي و مضغوط) 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 1-3 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 .**

Cl.tetani (TETANUS) :

Pathogenesis :

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.

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

Prevention:

by vaccination

Diagnosis:

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

Clostridium Botulinuim

- **Found in soil** ponds and lakes

- 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

- **Botulism** (يعني التسمم من هذي البكتيريا)
  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 يعني باختصار إذا ما تم طهيها بشكل جيّد راح تكوّن سبورز وتنتج توكسين

- 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 week 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 sever cases +Penicillin

- Prevention

  Adequate pressure cooking autoclaving and heating of food for 10 minutes at 100 OC

Clostridium Difficile

* Normal flora in gastrointestinal tract after exposure to antibiotics and killing of other normal flora, this organism will multiply witch then produce toxin that has two components:

a. Subunit **enterotoxin** (cause diarrhea)

b. Subunit **Cytotoxic** (kill the cells i.e. necrosis)

* Pseudomembrane colitis 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)**

Clostridium Difficile

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.

Questions

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- An example of a gram positive bacili:

A- Clostridia B- Peptococcues C- Actinomyces

6- What bacteria is always resistant to penicillin:

Ans:…….

7- Veillonella parvula is always in:

A- Gram negative cocci B-gram positive cocci C-gram positive cocci in clusters

8- Cl.tetani is prevented by:

Ans:….

9- What cuses toxic enterocolitis :

Ans : …..

Answers

1- A

2- B

3- B

4- T

5-C

6- bacteroides fragilis

7-A

8- vaccination

9- cl.difficle (pseudomembernous colitis)

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