MEDICINE
KING SAUD UNIVERSITY

microbiology

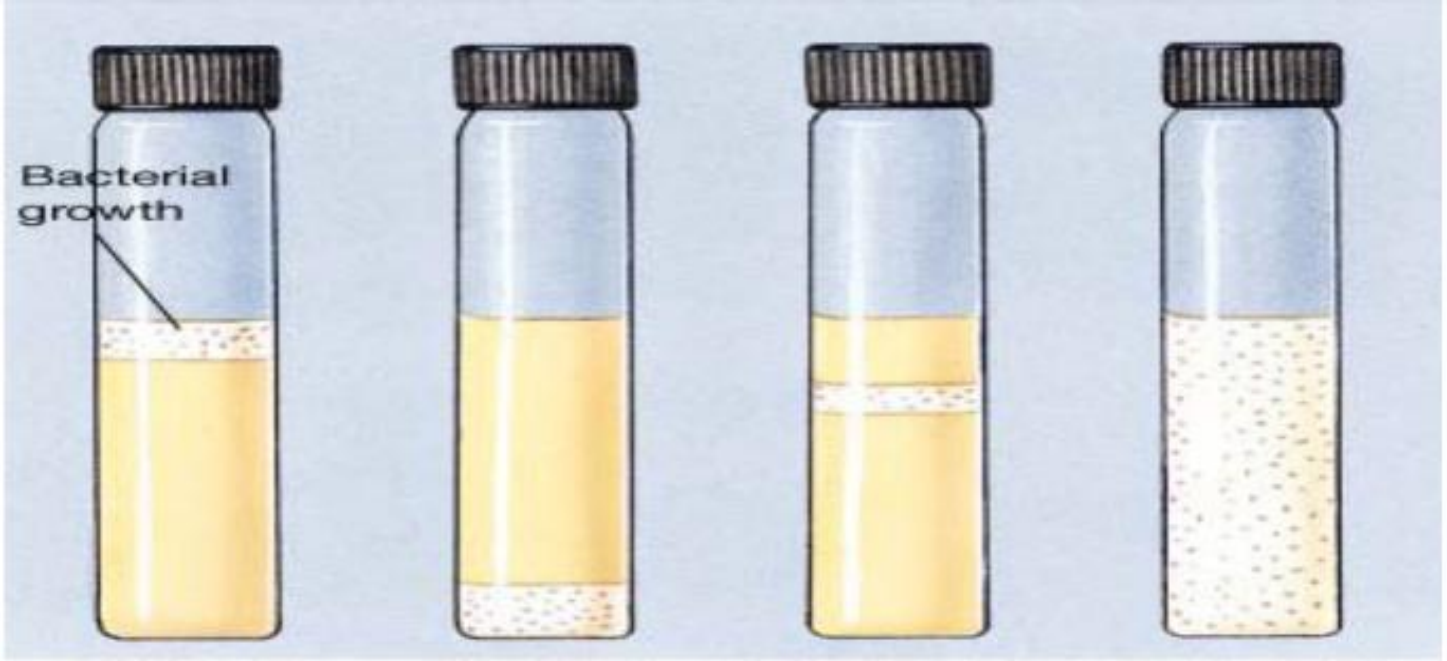
LECTURE: ANAEROBIC

IMPORTANT.
DOCTORS NOTES.
EXTRA INFORMATION.

ملاحظة مهمة جداً :
هذه المحاضرة معتمدة على سلايدز الدكتورة وتيم الأولاد

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.
- ✓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.



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 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 .***

Anaerobiosis

Only in female's slides

DEFENITION:

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

- 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
- They lack an enzyme called superoxide dismutase.

Superoxide O2- are similar to free radical
- Highly toxic
- Highly reactive

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

HABITAT

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)* ***Bacteroides fragilis*** *(2) Bifidobacterium species*

C. Female genital tract (mainly in the vagina)

-presence as normal flora also in Skin, Nose, Mouth, throat, Endocervix, Urethra.

Modefied by:

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

المقصد إنها تحول البكتيريا من نورمال فلورا الى ممرضة

CLASSIFICATION

❖1-SPORE FORMING

GRAME POSITIVE BACILLI

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

❖2-NON SPORE FORMINGN {MORE COMMON}

A –GRAM NEGATIVE **BACILLI**

- **bacteroides fragilis** (resistant to penicillin)
- Prevotella spp
- Leptotricha buccalis
- **fusobacterium spp** (The main cause of pharyngitis)
- f.nucleatum
- **Viellonella sp.** **GRAM NEGATIVE DIPLOCOCCI**

B –GRAME POSITIVE ***COCCI***

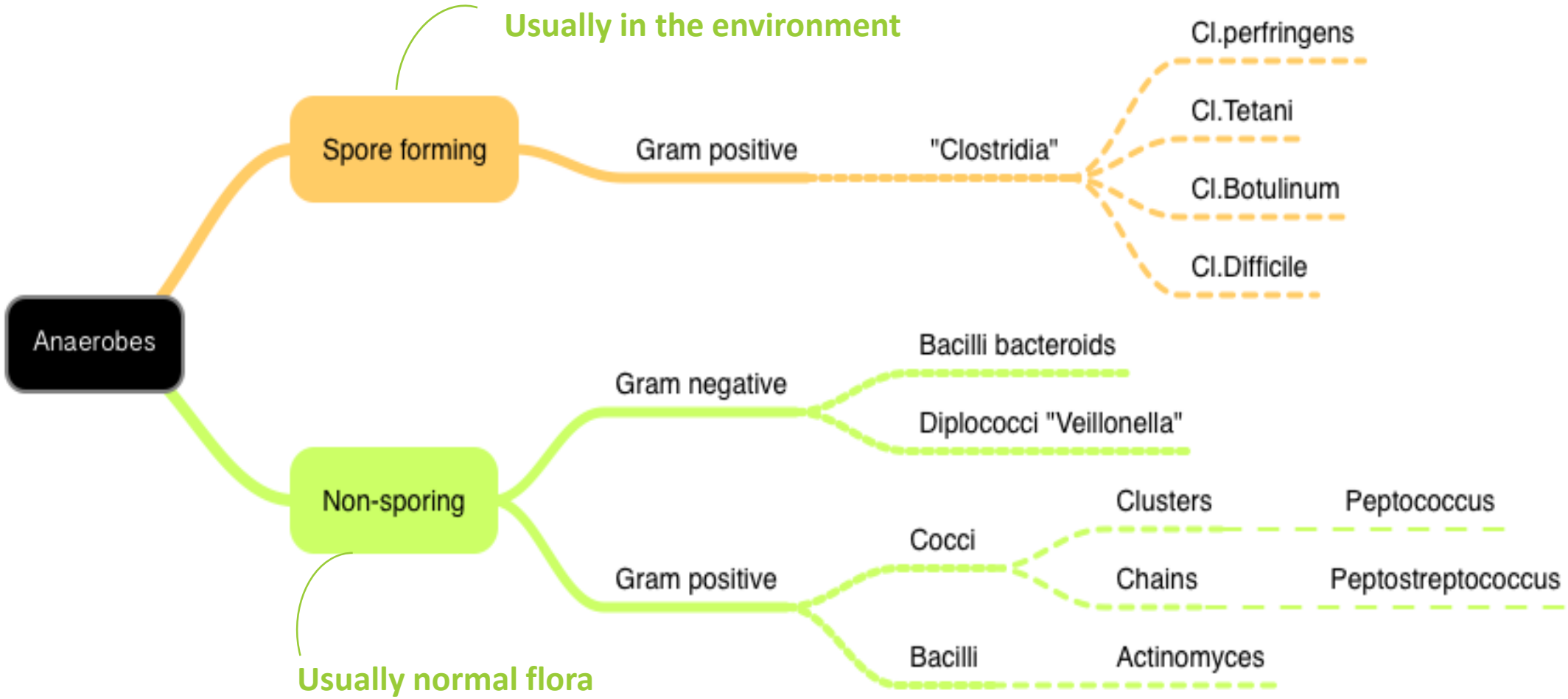
- **Peptococci** (staphylococcus)
- **Peptostreptococci** (streptococci)

C –GRAME POSITIVE **BACILLI**

- Propionobacterium propionicum ,p.acne
- Bifidobacterium
- Euobacterium
- LACTOBACILLUS
- **Actinomyces** israelii

D-MICROAEROPHILIC STREPT

Classification of anaerobics:



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

Anaerobic bacteria

Non spore forming

Gram positive

Bacilli

- Actinomyces causing : actinomycosis
- Propionibacterium causing : acne
- Mobiluncus causing : bacterial vaginosis
- Lacto bacillus causing :endocarditis
- eubacterium
- bifidobacterium

cocci

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

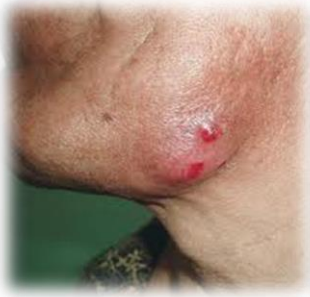
Gram negative

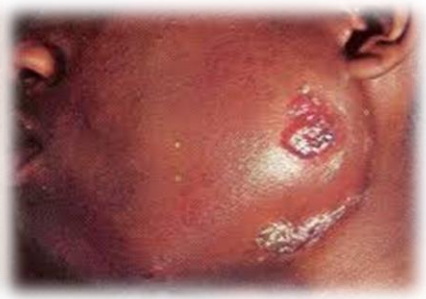
Bacilli

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

cocci

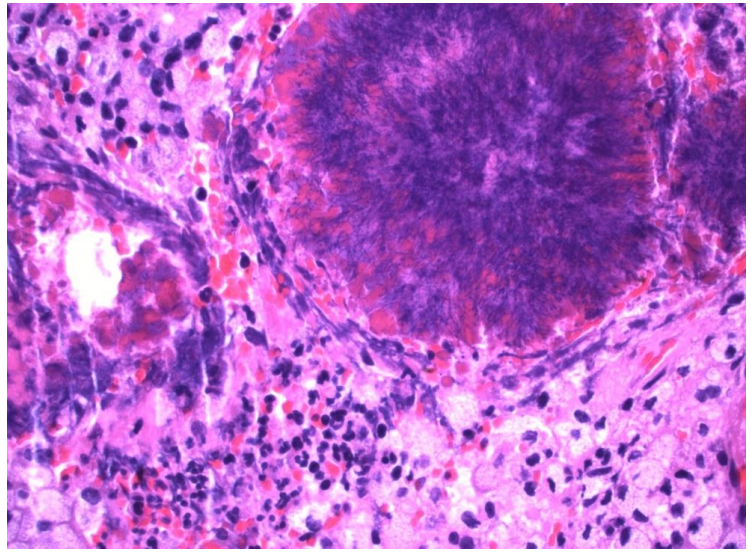
- Veillonella (diploocci)
  The only one





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

ACTINOMYCES SPP.



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

osteomyelitis

Pathogenesis

Abscess

After tooth extraction the normal flora invade the soft tissue

Extra info
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.

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 and many more -no pigmentation of colonies or fluorescence	-bile sensitive -resistant to kanamycin only -some pigmented

Treatment : metronidazole (flagyl) is the drug of choice.
Clindamycin can also be used.

EPIDEMIOLOGY:

Almost all infections are indigenous except:

- Tetanus
- Infant ,wound botulism
- Bites
- C .difficile {nosocomial (acquired or occurring in a hospital) }
- Gas gangrene { some cases }

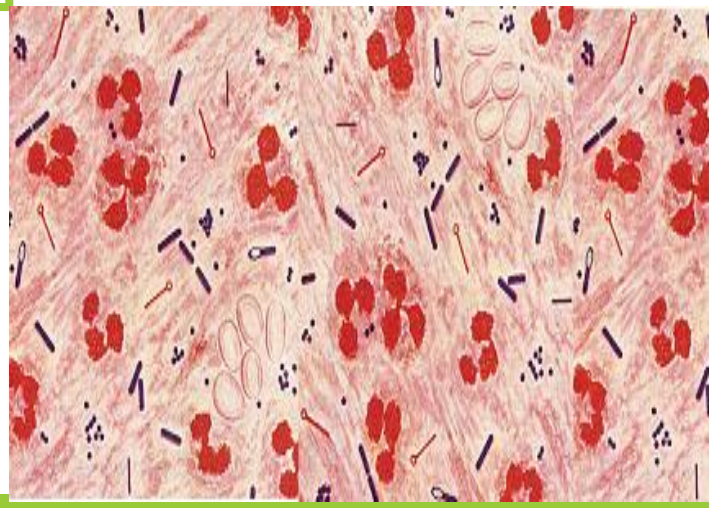
FEATURES OF ANAEROBIC INFECTIONS:

- Infections are always near to the site of the body which are habitat.
- Infection from animal bites.
- Deep abscesses
- The infections are also polymicrobial
- 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.

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

IMPORTANCE:

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



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
- Cholecystitis
- Obstruction
- Ulceration
- Diabetes mellitus
- Pylephlebitis
- Diverticula formation

HOW DOES THE INFECTION BEGIN ?

-DISRUPTION OF BARRIERS	-DISRUPTION OF BLOOD SUPPLY
Trauma	Drops oxygen content of tissue
Operations	Decrease in eh potential
Cancerous invasion of tissues	Tissue necrosis

WHAT ARE THE INFECTION CAUSED BY THESE ANAEROBIC ORGANISMS :

- ❖ Post operative wound infection.
- ❖ Brain, Dental, Lung abscess.
- ❖ Intra-abdominal abscess, appendicitis, diverculitis

All these infection can cause bacteremia

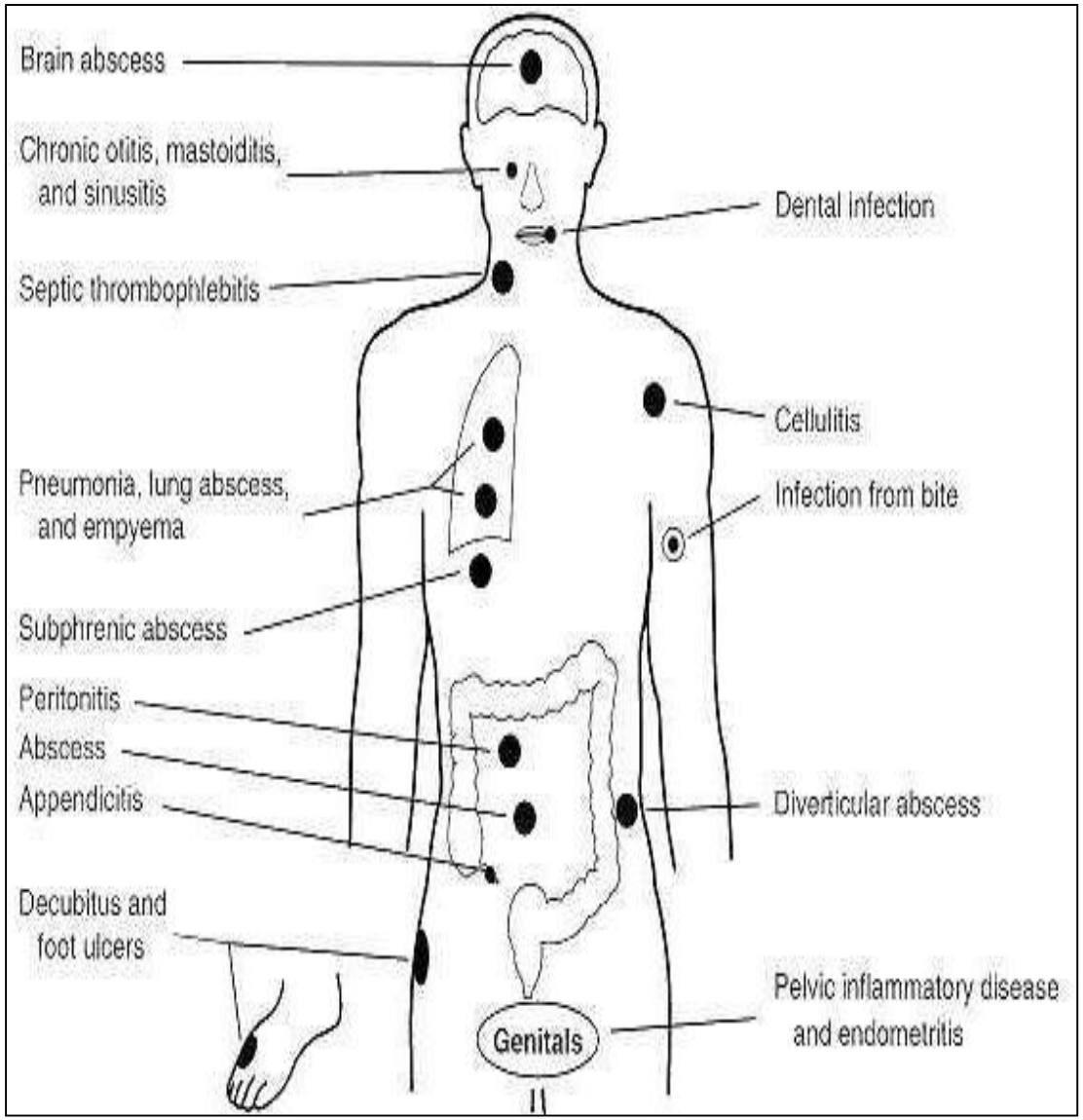
- ❖ Infection of the female genital tract
- ❖ Septic abortion
- ❖ Puerperal infection or sepsis
- ❖ Endometritis
- ❖ Pelvic abscess

Other infections

- a) Breast abscess in puerperal sepsis
- b) Infection of pilonidal sinus
- c) Infection of diabetic patients (diabetic foot infections).

Frequency with which Anaerobes are Associated with Human Disease

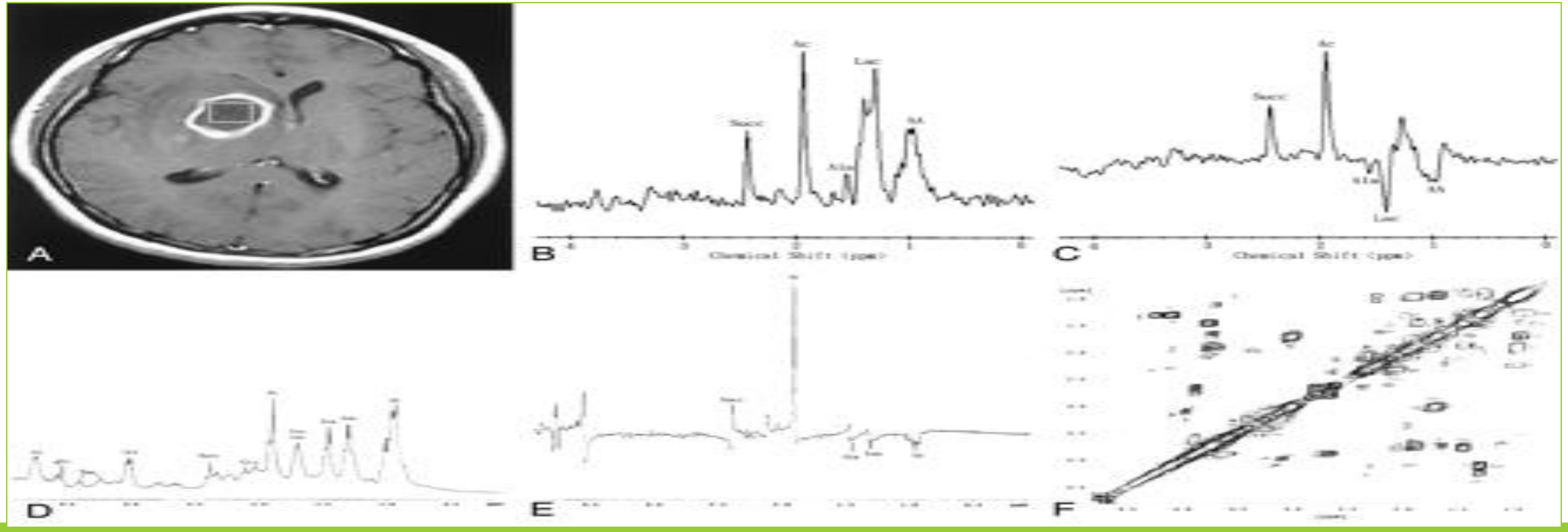
Infection	Anaerobes Involved (%)*
Bacteremia	10-20
Central nervous system	
Brain abscess	89
ENT-dental	52
Thoracic	
Aspiration pneumonia	93
Lung abscess	93
Empyema (nonsurgical)	76
Intraabdominal	
General infection	86
Liver abscess	50-100
Appendicitis	96
Obstetric-gynecologic	
Vulvovaginal abscess	74
Pelvic abscess	92



Only in female's slides

Only in female's slides

- A 43-year-old man with surgically proved pyogenic brain abscess in the right basal ganglion secondary to *Eubacterium lentum* (obligate anaerobe) infection.
- Axial contrast-enhanced T1-weighted MR image shows a ring-shaped cystic lesion and surrounding edema.



LABORATORY DIAGNOSIS:

When anaerobic infection is suspected:

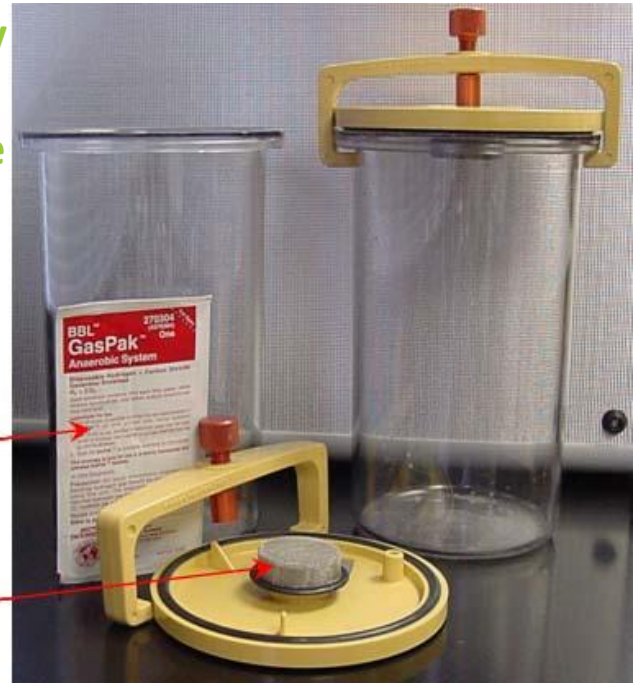
- ✓ Specimens have to be collected from the site containing necrotic tissue.
- ✓ Pus is better than swabs.
- ✓ Specimens has to be send to the laboratory within 1/2 hour why?
- ✓ Fluid media like cooked meat broth are the best culture media.
- ✓ Specimens have to incubated anaerobically for 48 hours.

Because they are slowly growing pathogens

Because they are anaerobic so they need immediately special environment without O2 to survive

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.



Clostridium Species

- **Morphology:** - Large gram positive rods **-Spore forming.**

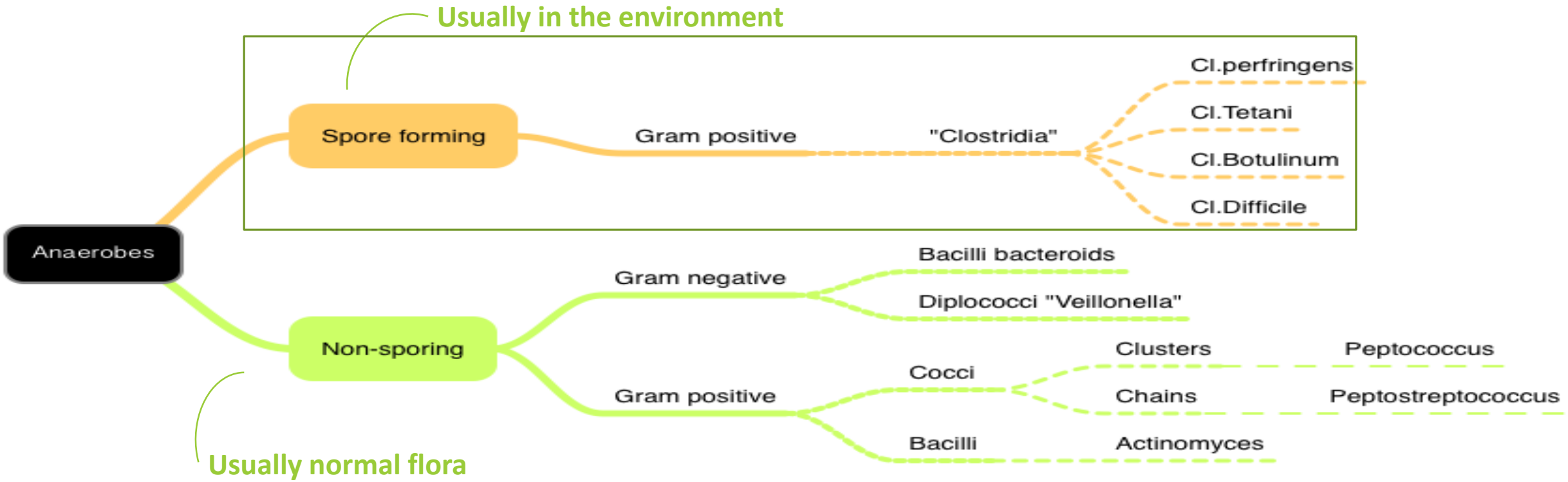
❖ **Causative agents (مسببة) for:**

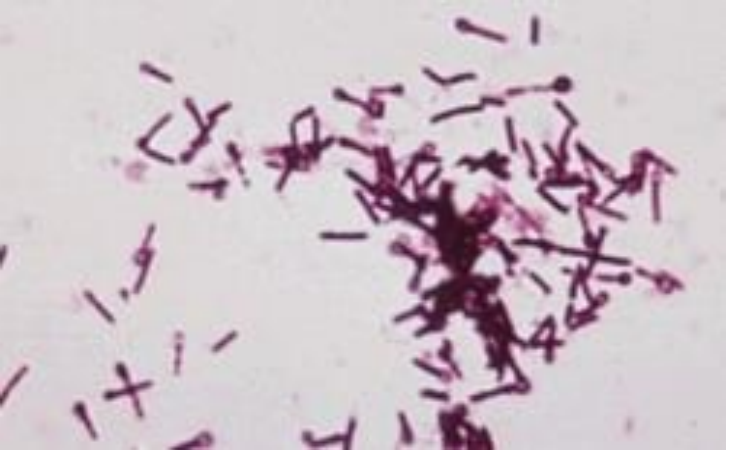
1.Gas gangrene : Cl. perfringens and other e.g septicum.

2.Tetanus : Cl. tetani

3.Botulism : Cl. Botulinum

4.Toxic enterocolitis : Cl. difficile (Pseudomembernous colitis)





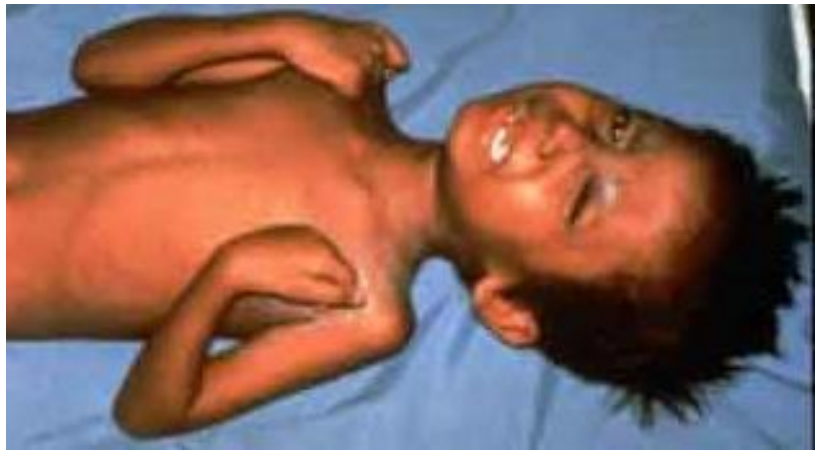
TETANUS

TRIMUS

LOCKJAW 1884

Strict toxigenic disease

Other name for this disease



Cl.tetani (TETANUS)

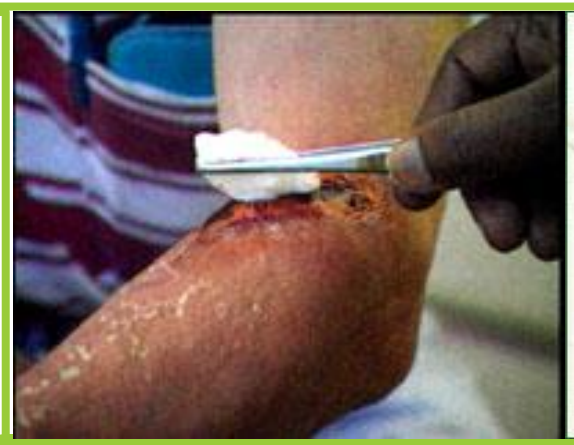
-Morphology gram +ve bacilli anaerobic with terminal spore. Drum Stick appearance

-Face & neck and upper extremities wounds are more dangerous

- SOURCE : **Lives in soil and animal feaces. e,g horse and any wound can infected if contaminated by spores**

Other sourse

- **Contaminated wound {minor}**
- **Compound fracture**
- **Narcotic addicts**
- **Unsterile injections**
- **Burns , bites ,avulsions**
- **Umbilical stump**



Clinical Features

- Incubation period 1-3 weeks= $3\text{-}21\ \mathrm{days}$(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)**
- **Arching 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 .**

EPIDEMIOLOGY:

- 1 Million/year > 60 yr .injection of drugs {young}
- ½ due to neonatal tetanus
- Cryptogenic t. {23%}
- Disease of non-immunized animals and humans {toxoid}

Pathogenesis:

- EXTOXIN {TETANOSPASMIN}
- Presynaptic terminals of LMN Inhibitory impulses to MNs (motor neurons)
- Persistent tonic spasm

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 especially lower motor neurons at anterior horn cells of cord.

Diagnosis:

Mainly by clinical.

Laboratory , and it is strict anaerobe very motile spread on agar{minor role}

Prevention:

(by vaccination)

Complete active childhood immunization

Appropriate wound management

Type of wound

Immunization history

Treatment:

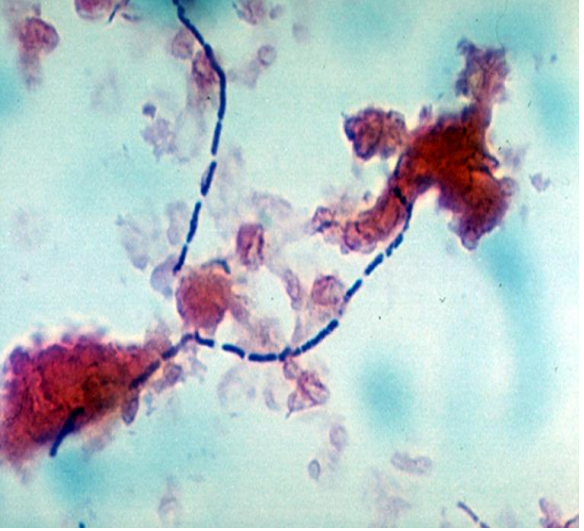
- Supportive
  Airway or Muscle relaxant or Wound care
- Antitoxin : TIG {500 UNITS}
- Antibiotics :MTZ , PG , penicillin

Cleaning of wound and removal of Foreign body (supportive)

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

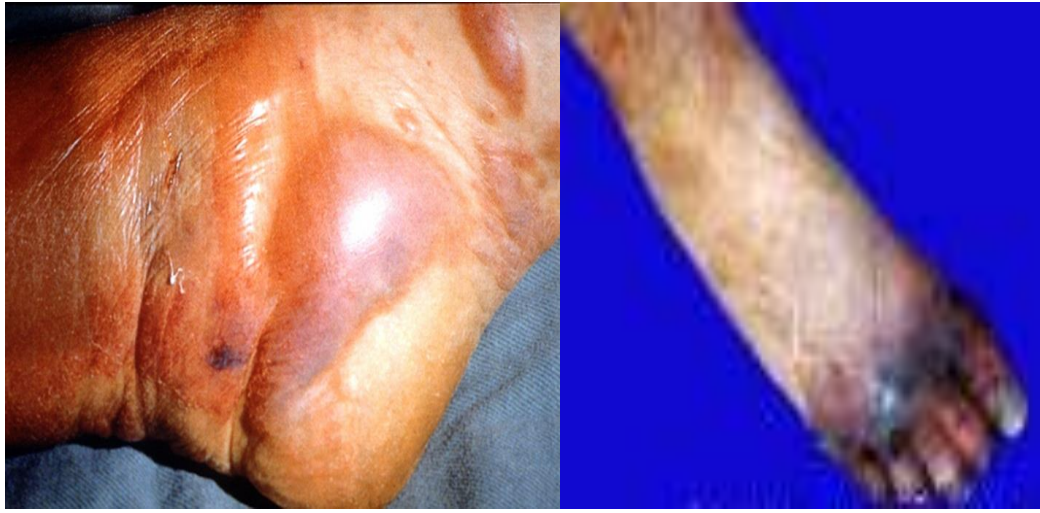


C. Perfringens {C.Welchii}

Histotoxic clostridia

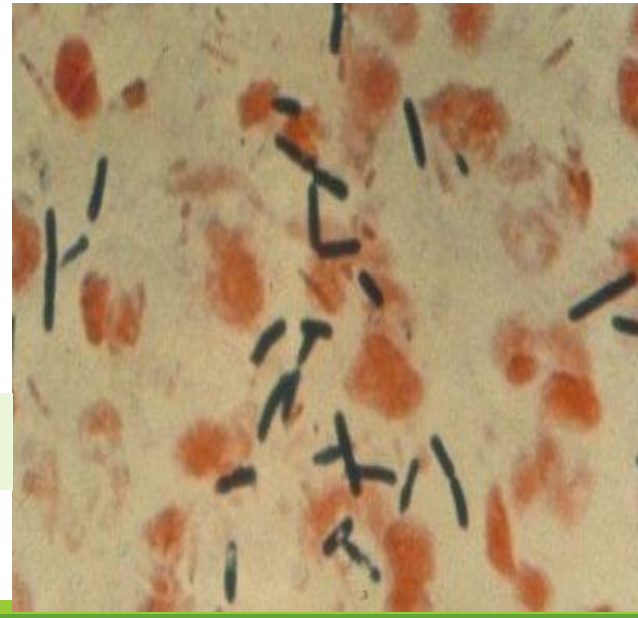
Gas gangrene

Food Poisoning



NATURAL HABITATS:

- Soil and intestinal tracts of animals and humans { 10^3 10^8 }
- Widespread occurrence
- Vagina of 1-9 % healthy women (as normal flora)



-**Morphology**: large rods gram +ve with bulging endospores subterminal.

DIAGNOSIS:

- CLINICAL
- SURGICAL
- MICRO. Gram + bacilli

-Gram stain :- G PB , absent leukocytes

-Culture { aerobic and anaerobic }

- Exudate , aspirates
- Tissue
- Blood

◦ Nagler reaction

Laboratory diagnosis:

Smear Gram stain Large Gram positive bacilli with few or no WBCs.
-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.
المقصود ان اختبار ناقلار يفرق بين البيرفرنجنس و غيرها من الكلوستريديم وهو يستعمل فيه صفار البيض

Cooked meat medium →

CLINICAL SIGNIFICANCE

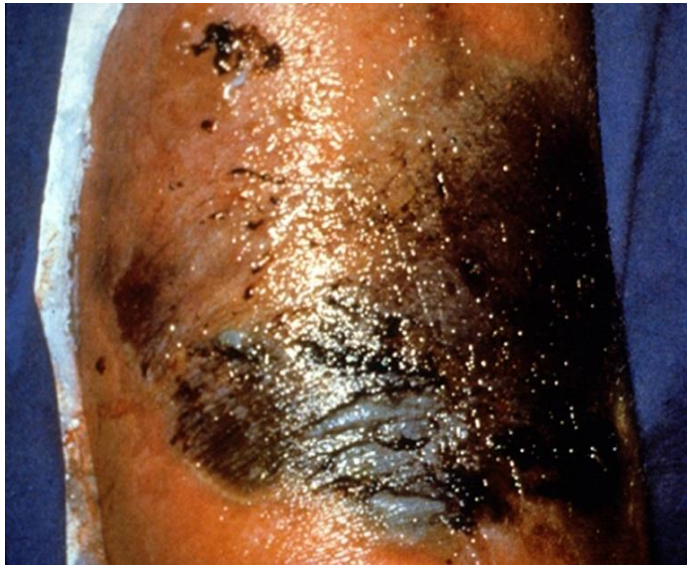
- Species most commonly isolated from clinical specimens for this disease.
- Many clinical settings ranging from :-
  - Simple contamination of wounds – traumatic or non traumatic myonecrosis
  - C. Cellulitis
  - Intra-abdominal sepsis
  - Gangrenous cholecystitis
  - Post-abortion infections – septicemia
  - Bacteremia
  - Brain abscess

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

Gas gangrene

- Toxin mediated breakdown of muscle tissue
- Rapid progression { like Uterus }
- Liquefactive necrosis of muscle , gas formation , toxemia.
- Fulminant septicemia
- Intravascular hemolysis
- Hemoglobinuria
- Blood cultures positive in 15 % of patients



Clinical picture

- Acute progressive pain , edema , skin discoloration
- Systemic – fever , tachycardia , hypotension , renal failure , crepitus , pulmonary edema , death

ETIOLOGY

- **C.perfringens** { 80% }
- C.Novyi
- C.Septicum
- C.Histolyticum

Pathogenesis:

Traumatic open wounds or compound fractures
(كسر يخترق الجلد و يسبب انفيكشن)
lead to muscle damages and contamination with dirt .
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

5 – Toxins [A – E] لها خمس أنواع من السموم
Phospholipase C { alpha toxin } واحد من هالأنواع
Acts on membranes of muscle cells , leukocytes and platelets
Play major role in the pathogenesis of C.myonecrosis
Has necrotizing activity
Other toxins :- collagenase , proteinase , DNAs

TRATMENT:

- Early and complete surgical excision of necrotic infected tissue { most important }
- High dose of :-
  - Penicillin G IV
  - Metronidazole
  - Clindamycin
- Management of shock , hemolysis , anemia

Prevention and Treatment:
Remove dead tissue , debris and foreign bodies .Penicillin and hyperbaric oxygen (اعطاء المريض اوكسجين نقي و مضغوط) in some cases.

FOOD POISONING:

- One of most common bacterial causes of food –borne illness
- Sporadic cases and outbreaks
- Almost all due to type A
- Improperly cooked meat or meat product
- Ingestion of vegetative cells [108]
- Afebrile Crampy abdominal pain - diarrhea within 7-15 h
- Enterotoxin [SPORULATION]
- Mild illness , recovery after 2-3 days

C.BOTULINUM

BOTULISM

- **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

TRANSMISSION

SPORE → VEGETABLES , MEATS ,FISH → CANNED FOOD→ PREFORMED TOXIN

PATHOGENESIS - TOXIN (PHAGE) - MOST TOXIC SUBSTANCE

Ingestion of Spores → germination in the GUT → BLOOD → PERIPHERAL NERVE SYNAPSES → BLOKS RELEASE OF ACETYLCHLINE→ FLACCID PARALYSIS

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

CLINICAL:

- DESCENDING PARALYSIS
  - DIPLOPIA
  - DYSPHAGIA
  - RESPIRATORY MUSCLE FAILURE
- NO FEVER
- WOUND , INFANT BOTULISM (honey)

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

• Laboratory Diagnosis:

- clinical (TOXIN ,FOOD SERUM)

  Suspected food from the patient **Faeces Culture** or serum toxin detection

• Treatment :

- ANTITOXIN : Mainly supportive and horse antitoxin in sever cases
- A , B ,E (types of antitoxin)
- RESPIRATORY SUPPORT

• Prevention :

STERILIZATION OF CANNED FOOD

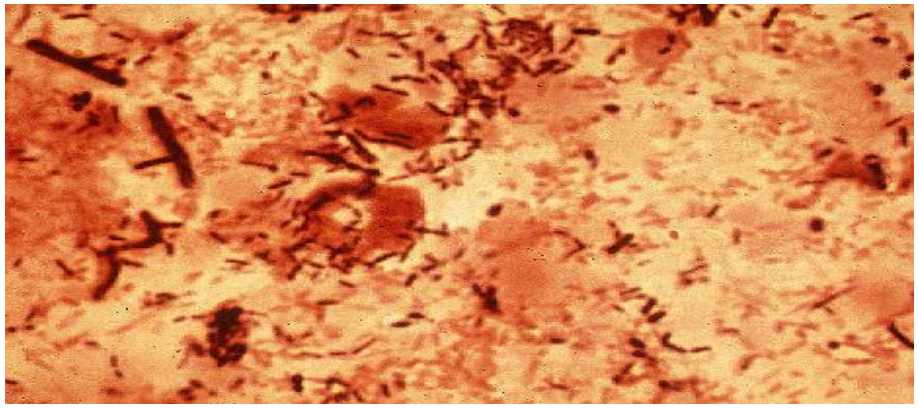
(Adequate pressure cooking autoclaving and heating of food for 10 minutes at 100 OC)

C . Difficile

Pseudomembranous colitis

Antimicrobial associated diarrhea

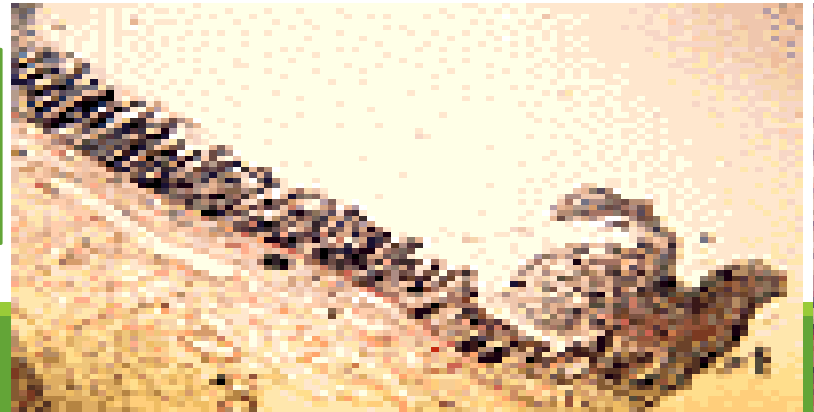
Hospital acquired diarrhea

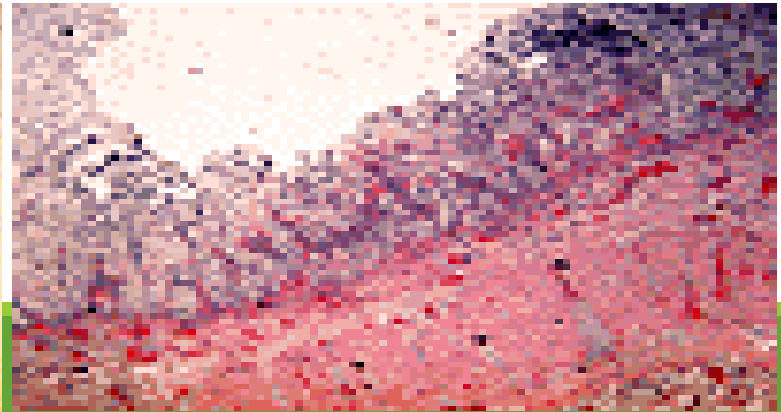


EPIDEMIOLOGY:

- Overgrowth of Clostridium difficile in the colon, usually after the normal flora has been disturbed by anti microbial chemotherapy
- Clostridium difficile causes antibiotic associated diarrhea (AD) and more serious intestinal conditions such as colitis and pseudo membranous colitis .

Pseudomembrane is the clinical manifestation of this disease which composed of bacteria , fibrin , WBCs and dead tissue cells





EPIDEMIOLOGY:

- Sourse :
- Soil
- Human and animal feces
- Hospital environment {Reservoirs}
- Spores acquired
  - Environment
  - Fecal – oral { colonized persons }
- Intestinal colonization rate
  - Healthy neonates , young infant [50 %]
  - Children > 2yrs , adults {3 % }

CLINICAL PICTURE

- Mild diarrhea , asymptomatic carriage

– Toxic megacolon ,bowel perforation and death

- Pseudomembranous colitis
  - Bloody diarrhea , abdominal cramps,
  - Fever , systemic toxicity
  - Colonic mucosa – yellowish plaques
- Sever disease – neutropenic , inflammatory bowel disease

Severe dehydration , intestinal obstruction and perforation are some of complication of this syndrome.

PATHOGENESIS

- TOXINS that has two components:
  - a. Subunit TOXIN A (**enterotoxin**) cause diarrhea.
  - b. Subunit TOXIN B (**Cytotoxic**) kill the cells i.e. necrosis and is more potent
- . Most strains produce both or no toxins

DIAGNOSIS

- Endoscopy : pseudomembranes and Hyperemic rectal mucosa
- Stool : toxins { EIA } , Cell culture to Confirm toxigenic strains
- Isolation of C. Difficile { not diagnostic }
- PCR (Polymerase chain reaction)

TREATMENT

- Metronidazole for 7-10 days , oral , IV
- Oral vancomycin : {emergence of VRE }
- 10-20 % relapse rate
- Antimotility drugs : contraindicated
- Antimicrobial therapy : severe toxicity , persistent diarrhea
- Discontinue antimicrobial therapy{clinical significant diarrhea or colitis}

Risk Factors

- Exposure to organisms
- Disturbed normal gut flora {proliferate – toxin}
  - Repeated enema
  - Prolonged NG tube
  - GI surgery
  - Bowel stasis
  - Antimicrobials : penicillins , clindamycin , Cephalosporins

Control Measures

- Proper hand washing {contact precautions}
- Limiting use of antimicrobial agents
- Isolation of patients with diarrhea
- Disinfection of pt. rooms

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.
- The negative type of 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 on anaerobic bacteria

- 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)

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

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 botulinuim

* 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* 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 severe cases.

Prevention

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

Clostridium Difficile

* Normal flora in gastroentestinal 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 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.

Questions

1- A / 2- B / 3- B / 4- T / 5-C / 6- bacteroides fragilis
7-A / 8- vaccination / 9- cl.difficle (pseudomembernous colitis)

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 employ to generate energy for growth. a) T b)F
5. An example of an anaerobic gram positive bacili non forming spores : 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 : ……

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