علم الاحياء المجهرية للممرضين (1)

قسم تمريض/ المرحلة الثانية/ مسائي

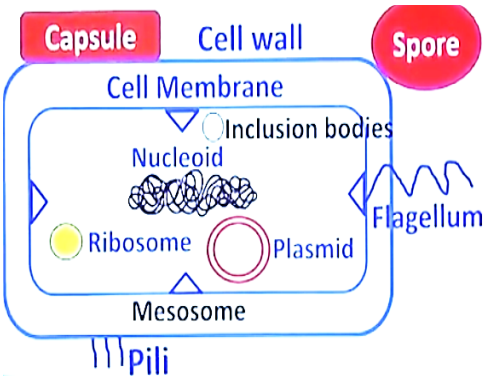
Dr. Sarah Abdulhussein Hasan

Lecture 1

Introduction to Microbiology and Bacterial structure

1. **Definition of Microbiology**
2. **Classification of Microbiology**

A. Eukaryotic
B. Prokaryotic
C. Viruses



3. **Bacterial structure**

A. Intracytoplasmic structure

1. Nucleoid
2. Ribosome
3. Inclusion granules
4. Cell membrane (cytoplasmic membrane) (mesosomes)
5. Plasmid

B. Cell wall

C. Structures outside the cell wall

1. Capsule
2. Flagella
3. Pili
4. Spore formation

1.Definition of Microbiology:- science that study organisms cannot be seen by naked eye (fungi, bacteria and virus)

- Unicellular
- Micro=small
- Bio= Life
- Logy= Science

2.Classification of Microbiology

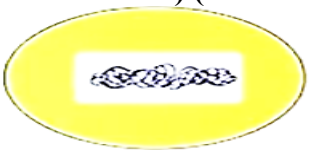
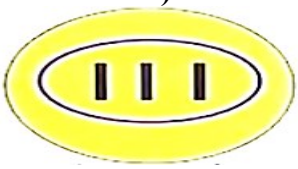
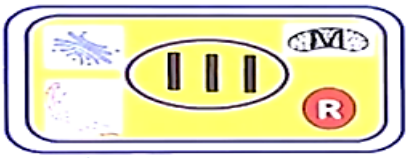
A. Eukaryotic (True nucleus)

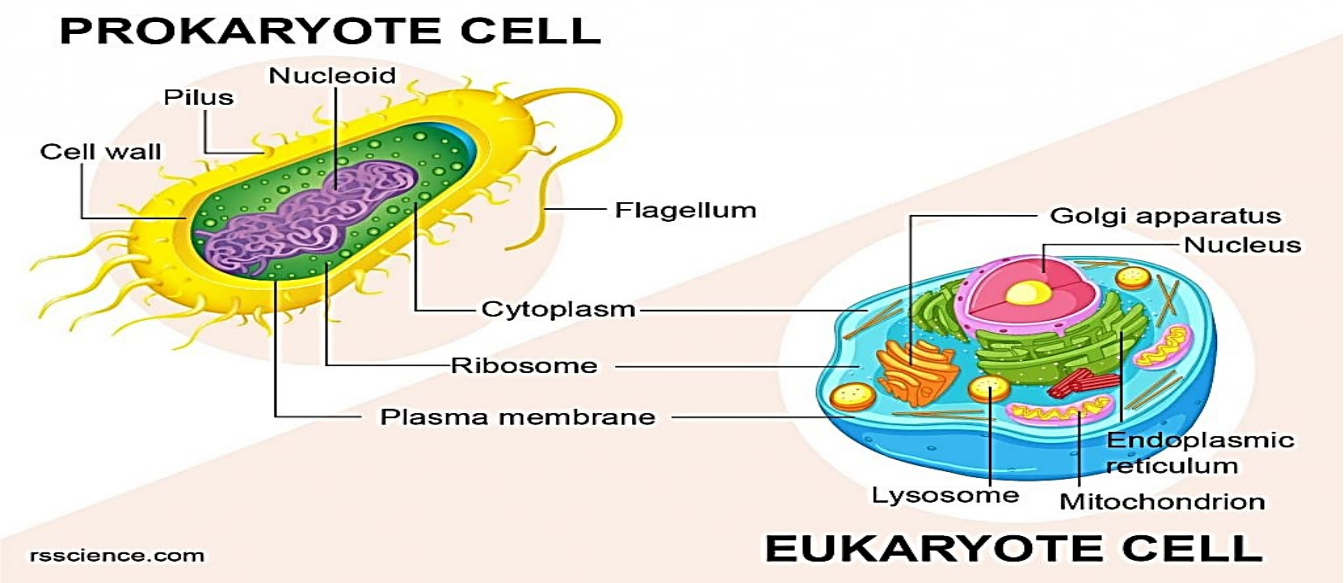
- Eu= True
- karyotic= Nucleus

B. Prokaryotic (No true nucleus)

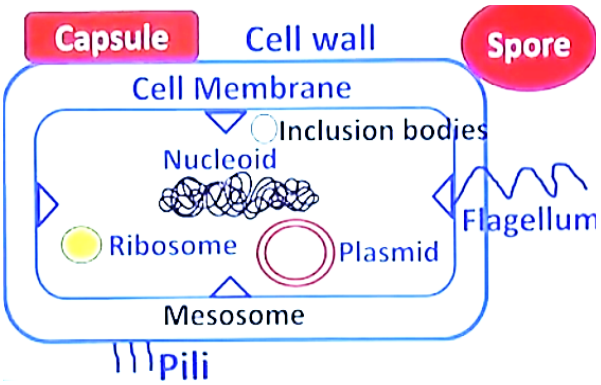
- Pro= Primitive
- karyotic= Nucleus
- Single chromosome (Suspended in cytoplasm (Nucleoid))

Compare between Prokaryotic and Eukaryotic

Characteristic	Prokaryotic	Eukaryotic
1) Nucleus	No (no true nucleus) (nucleoid) 	Yes (true nucleus) 
2) Size	0.05-10 µm	10-100 µm
3) Nuclear membrane	No (Nucleoid)	Yes (Nucleus)
4) Membrane bound organelles • Mitochondria • Golgi apparatus • Endoplasmic reticulum	Absent  ❖ Bacteria	Present  ❖ Fungi ❖ Protozoa ❖ Algae
5) Chromosome Number	One (circular)	Multiple (linear)
6) Ribosome	70S (30S-50S)	80S (40S -60S)
7) Cell wall	Present EXCEPT Mycoplasma	Absent Fungi (Chitin)
8) Cell membrane	No sterols EXCEPT In mycoplasma	Has sterols
9) Division	Binary fission	Mitosis



3. Bacterial structure



A. Intracytoplasmic structure

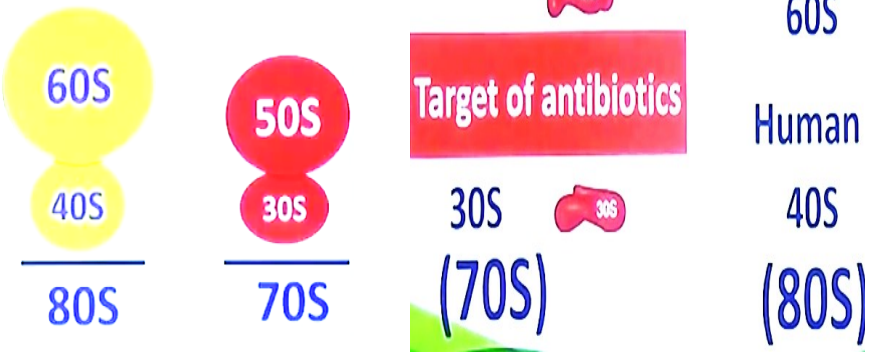
1) Nucleoid (Essential)

- Single chromosome
- Circular
- dsDNA
- 1mm in length
- Supercoiled
- Carry genetic information for growth & survival

2) Ribosome (Essential)

- Ribo=RNA
- Some=body
- Site of Protein synthesis
- Svedberg unit

1. 70S in prokaryotic (bacteria)
2. 80S in eukaryotic (human)



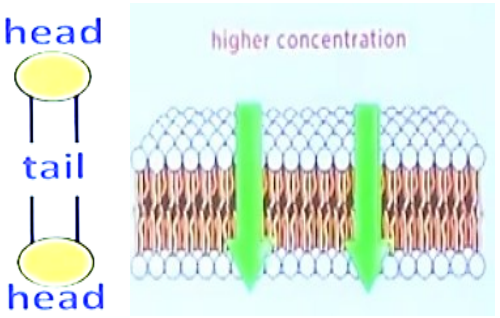
3) Inclusion granules

- Store of nutrient (Glycogen, Starch, Phosphate)

4) Cell membrane (cytoplasmic membrane)

A) Definition of the cell membrane (Essential)

- Thin, fragile membrane located just inside the cell wall



B) Composition of cell membrane

Phospholipid bilayer + Protein (**No sterols**)

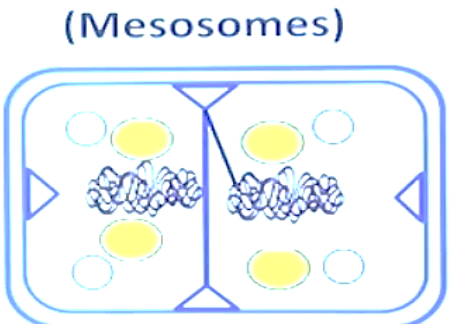
C) Function of cell membrane

1. Selective transport (Passive or active)
2. Mesosomes

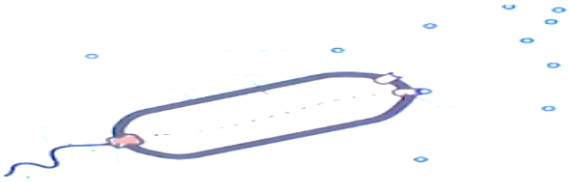
a) Respiration enzyme (Making energy)
(Like Mitochondria)

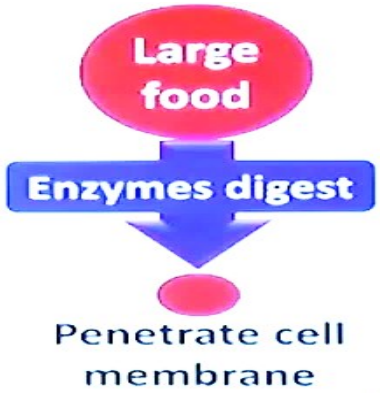
b) Cell division

- Separate DNA
- Septal mesosome

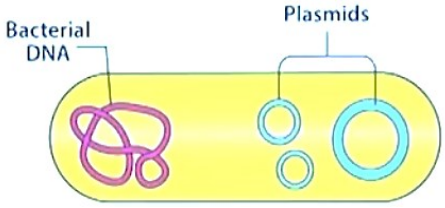


3. Biosynthesis of cell wall
4. Excretion of extracellular enzymes (Hydrolytic enzymes)
5. Excretion of extracellular enzymes (Penicillinase)
6. Chemotactic system (bacteria have flagellum)





5) Plasmid (Not essential):-



- **EXTRA** chromosomal dsDNA

Q/ why plasmid independent of bacterial chromosome

1. Replicate autonomously
2. Carry genes for toxin production and drug resistance

Harvesting questions

Q1) A prokaryotic organism has no

A. Cell membrane
B. Cell wall
C. Mesosomes
D. Nuclear membrane

Q2) One of the following is not function of cytoplasmic membrane

A. Respiration & Reproduction
B. Staining affinity
C. Selective transport
D. Cell wall biosynthesis

Q3) Mesosomes are:

A. Type of ribosome
B. A part of cell wall
C. Sites of respiratory enzymes
D. Type of genetic material

Q4) All of the following are characters of Bacterial ribosome EXCEPT:

A. Site of protein synthesis
B. 705
C. 80S
D. Target for antibiotic action

Q5) One of the following is not character of Plasmids

A. Extra dsDNA
B. Carry genes essential for growth
C. Carry genes for drug resistant
D. Replicate autonomously

Lecture 2

Bacterial Structure

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Bacterial structure

B. Cell wall

1. Definition
2. Composition
3. Synthesis (specially peptidoglycan)
4. Function
5. Cell wall deficient

1. Definition of Cell wall

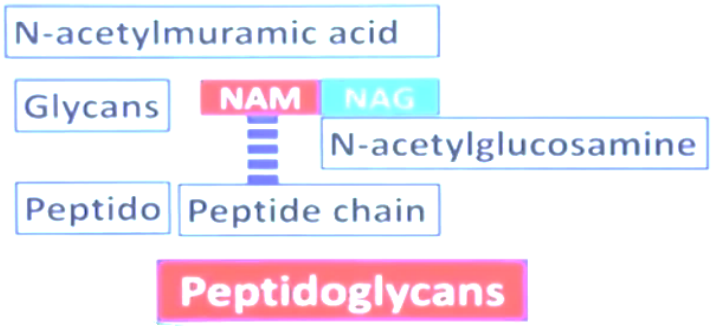
- Surrounds the cell membrane
- Rigid

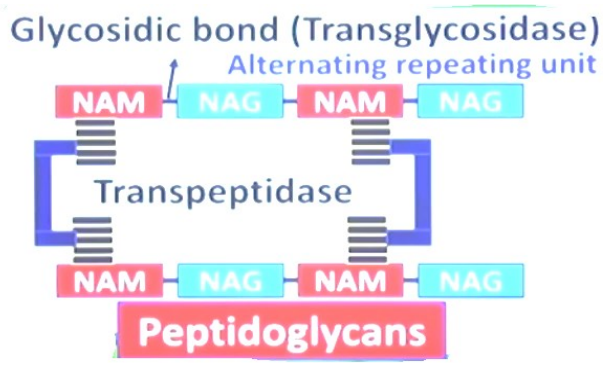
2. Composition of Cell wall

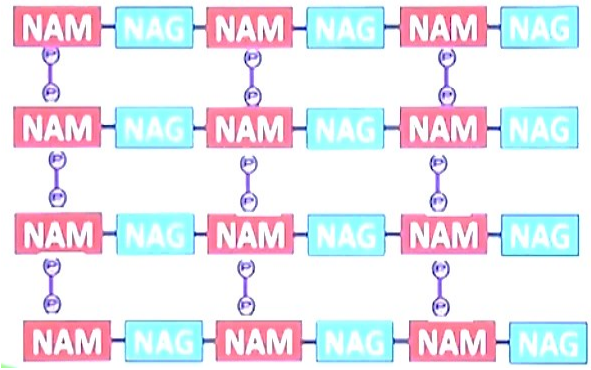
- Rigidity (Peptidoglycan)
    1. NAM (N-acetylmuramic acid)
    2. NAG (N-acetylglucosamine)
    3. Tetrapeptide side chain

C. Structures outside the cell wall (additional component of bacteria)

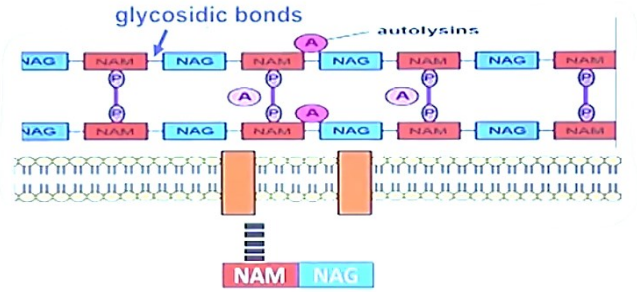
A. Capsule and related structure
B. Flagella
C. Pili (Fimbriae)
D. Spore formation



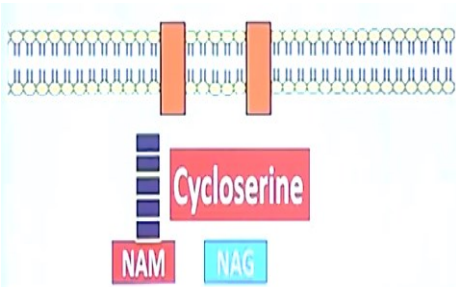




3. **Synthesis (Cell wall) (Peptidoglycan)**
   a) Formation of NAM-Peptide + NAG
   b) Break the glycosidic bonds by autolysin

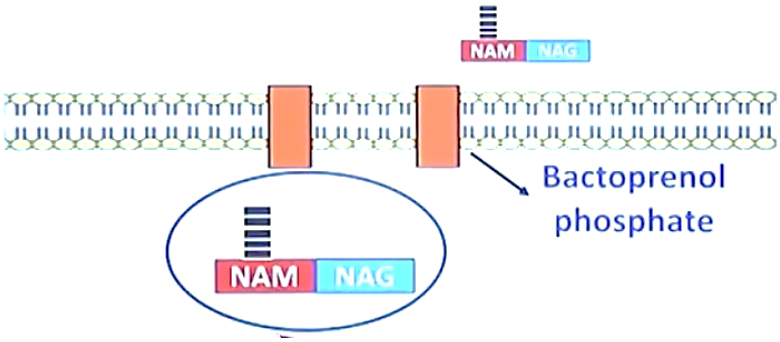


- **Cycloserine:-** antibiotic that inhibition cell wall synthesis by prevent break the glycosidic bonds by autolysin

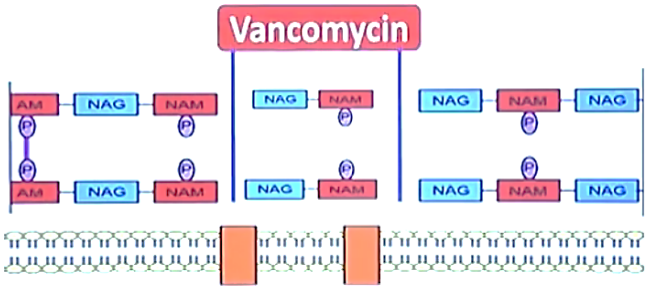


c) Bactoprenol transfer the unit outside cell membrane

- **Bacitracin:-** antibiotic that inhibition cell wall synthesis by prevent bactoprenol transfer the unit outside cell membrane (highly toxic that used only local)

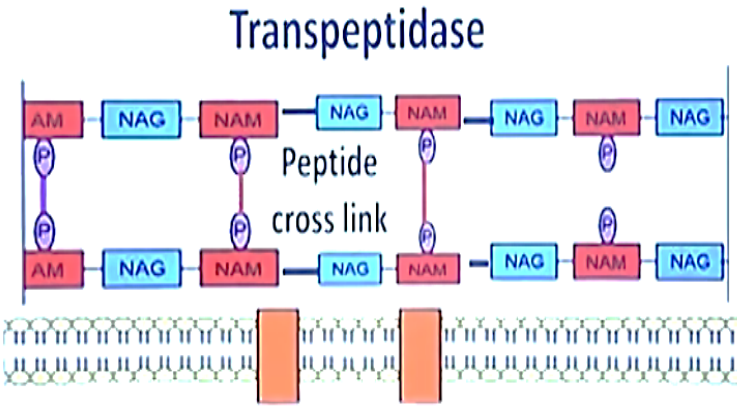


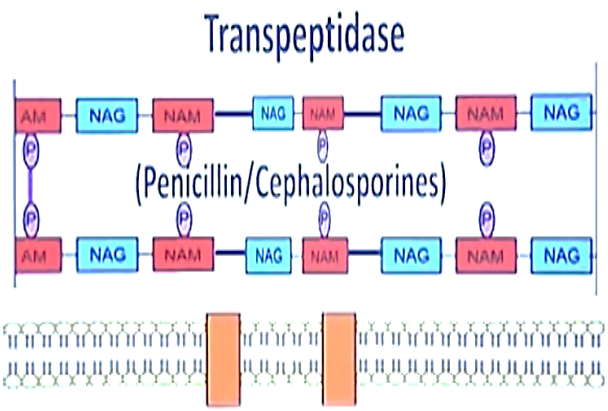
d) Transglycosidase (Glycosidic bond)
Vancomycin:- antibiotic that block transglycosidase



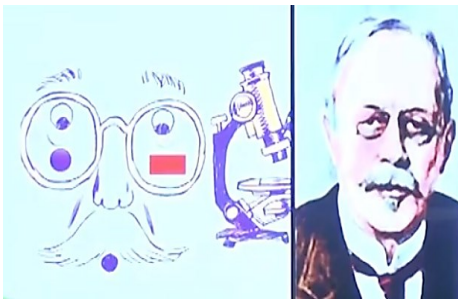
e) peptide cross-links (transpeptidase)

- **penicillin/ cephalosporines:-** antibiotic that block transpeptidase

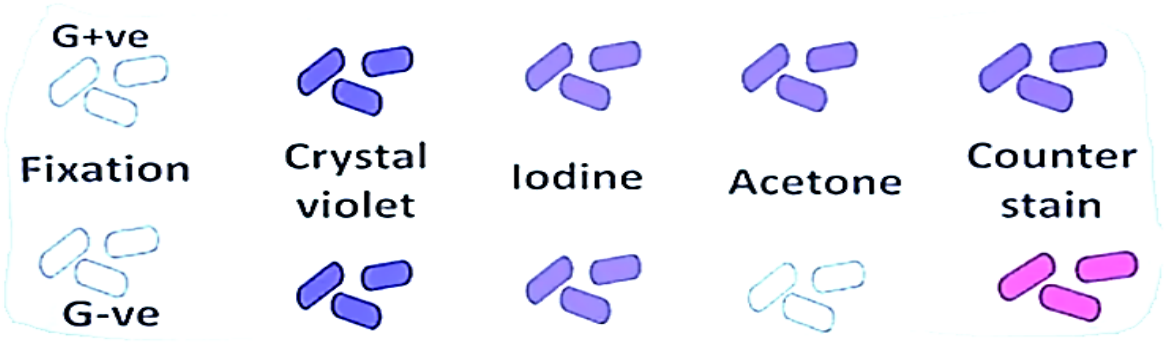


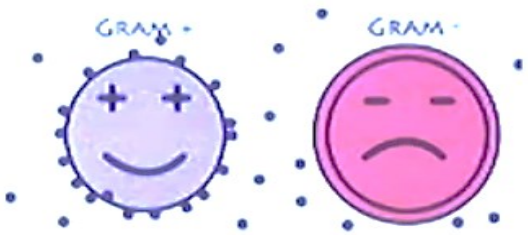


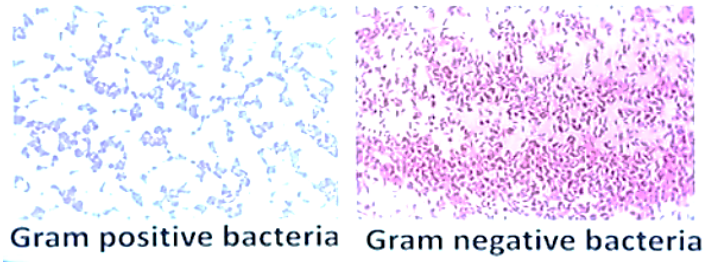
Q) Is penicillin destroy the complete bacterial cell?



❖ Gram scientist (Gram positive/negative bacteria)







Cell wall of Gram positive bacteria

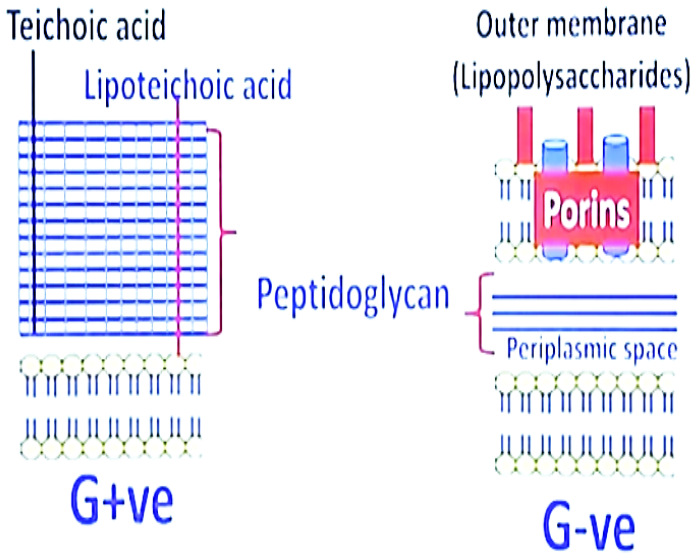
1) Peptidoglycan (50% thickness) (40 sheet)

- NAM- NAG
  Peptide
- Porous

2) Teichoic acid

- Polymers of glycerol or Ribitol
- Teichoic acid (Cell wall)
- Lipoteichoic acid (Cell membrane)
- Major surface Ag of G+ve
- Highly immunogenic (Toxic shock)

a) TNF-a
b) IL-1



Cell wall of Gram negative bacteria

1) Peptidoglycan

- A thin layer (5-10% thickness) (2 sheets of NMA & NAG)

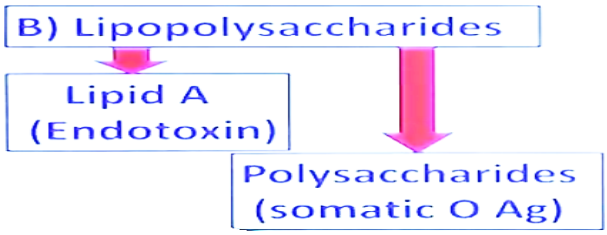
Peptides

2) Outer membrane

A) Bilayer phospholipids

B) Lipopolysaccharides

- Lipid A (Endotoxin)
- Polysaccharides (somatic O Ag)



C) Porins (hydrophilic Protein) in the outer membrane (Transportation)

3) Periplasmic space

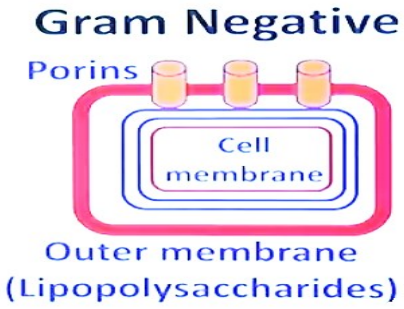
- Space between cytoplasmic & outer membrane
- Peptidoglycan layer & gel-like protein

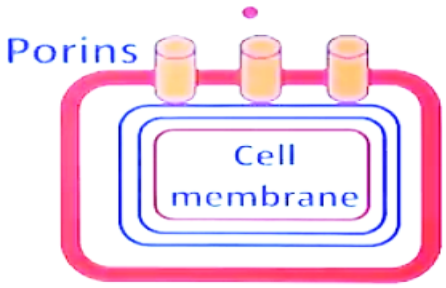
Q1)Compare between gram positive and gram negative bacteria according the cell wall

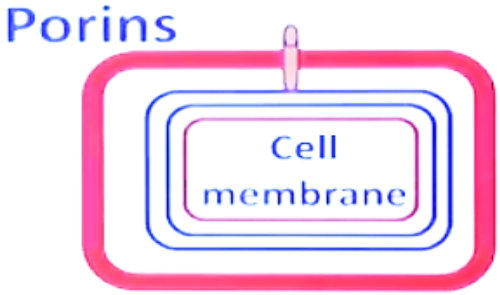
Characteristic	gram positive bacteria	gram negative bacteria
1)Peptidoglycan	Thick	Thin
2) Teichoic acid/ Lipoteichoic acid	Yes	No
3) Outer membrane	No	Yes
4) Porins	No	Yes

Q2) Many drugs are effective in gram positive than gram negative bacteria

- Penicillin G (Gram positive):- Large molecules and cannot pass on the porin of gram negative
- Ampicillin (broad spectrum) because it small molecule so can enter through porin
- Pseudomonas multidrug resistant because it has few porin and narrow or absent

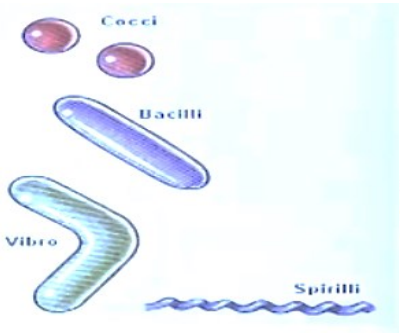






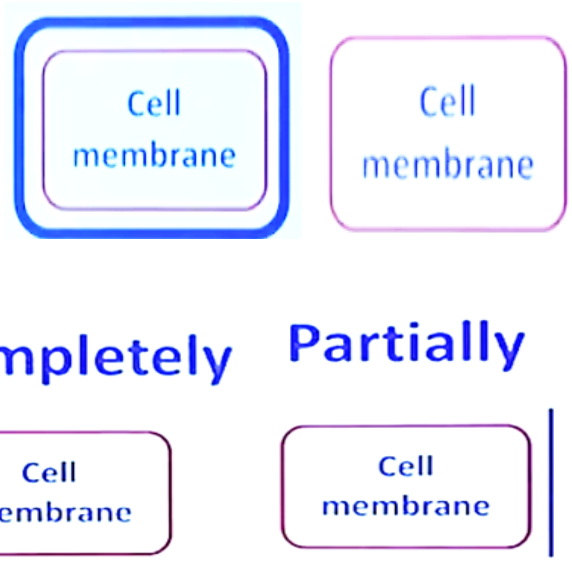
4. Function of cell wall

1. Maintenance of the shape (Rigid)
   - Deficient of cell wall (Polymorphic)
2. Protection (Osmosis insensitive)
3. Target site for antibiotics
   - Penicillin
   - Cephalosporines
4. Role in cell division
5. Responsible for staining



5. Cell wall Deficient:- bacteria without cell wall

1. **Naturally**
   - Mycoplasma (Sterol)
2. **Induced**
   - Cell wall inhibitors
   - Lysozyme
   - In Isotonic only
3. **Completely**
   - Protoplast (+ve)
   - Spheroplast (-ve)
4. **Partially**
   - L-form bacteria

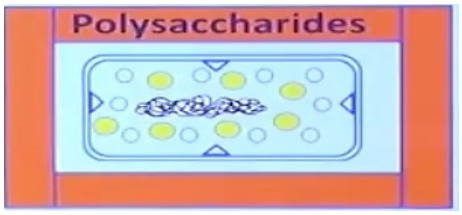


Notes

L-form & Mycoplasma:- resist to Penicillin & Cephalosporines

C. Structures outside the cell wall (additional component of bacteria)

A. Capsule and related structure

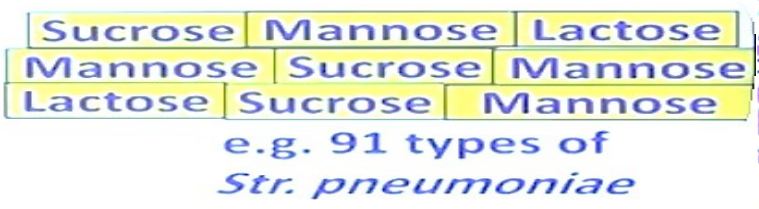


1) Capsule - Definition

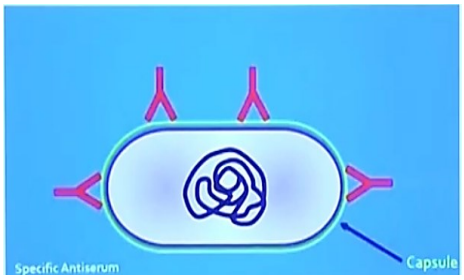
- Glyco/calyx (Carbohydrate / enveloped)
- Gelatinous (Viscous) layer covering cell wall of some bacteria

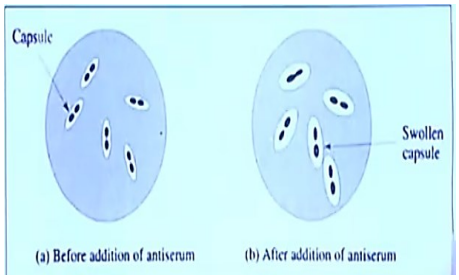
2) Capsule - Composition

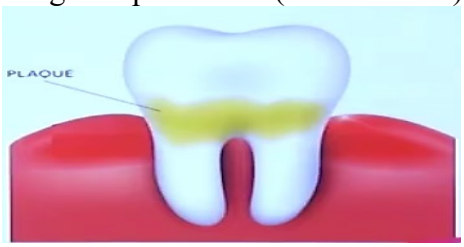
- Usually Polysaccharides
- Polypeptides (B. anthracis)
- Variation of Capsule (Arrangement of Polysaccharides)
- Do Not stained by Gram stain



- Quellung reaction (swelling)





Capsule	slime Layer	Glycocalyx
▪ Tightly, organized bound around all cell wall ▪ Well defines edges and borders 	▪ loosely adherents to surface of organism 	▪ Loosely & unorganized attached ▪ Fibrils extending ▪ It adhere firmly to skin, heart, etc ▪ e.g. Strept. mutans (Dental caries) 

3) Capsule - Function

1. A protect cell wall from
   - Bacteriophage
   - Complement
   - Lysozyme
2. Prevent phagocytosis (Virulence factor)
   - Capsules are formed in **VIVO ONLY**
3. Attachment (Glycocalyx) Dental caries
   - Attachment (Glycocalyx) (Prosthetic heart valves)
4. Development of vaccine

B. Flagella

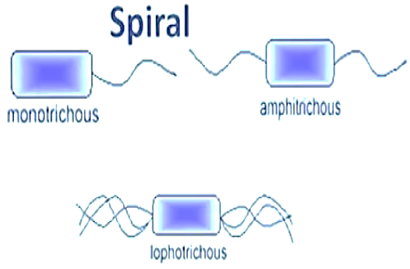
1-Flagella - Definition

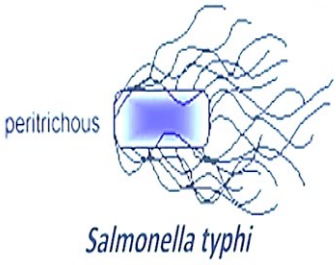
- Long thick threads like (filamentous), formed from protein (flagellin) (H Ag)
- Seen by EM (20nm)

2-Flagella - distribution

- Polar
- Spiral

1. monotrichous
2. amphitrichous
3. lophotrichous
4. Peritrichous (Around (Salmonella typhi))



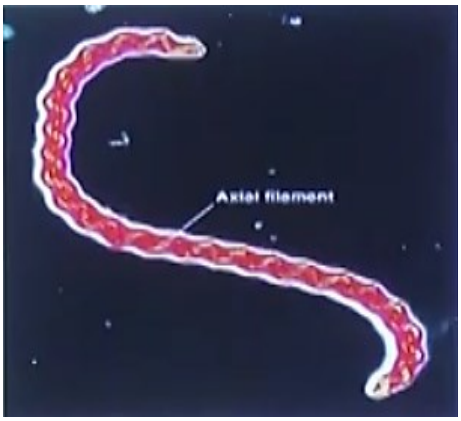


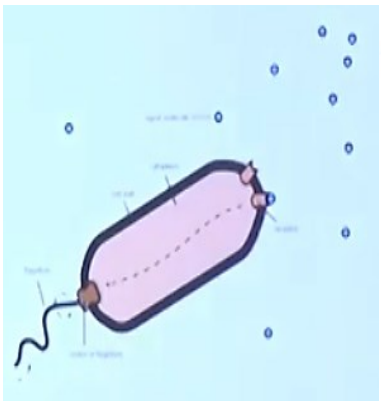
3-Flagella - Function

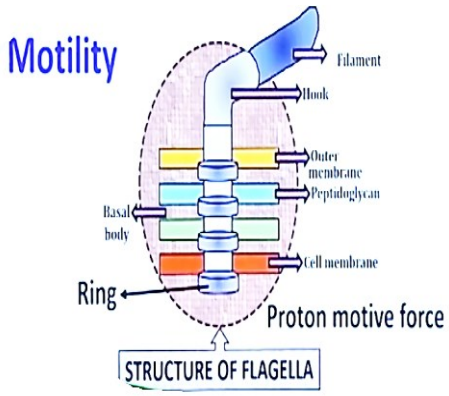
- The organ of motility
- Tactic response (Taxis) (Stimulus) (movement of bacteria to toward (+ve) or away (-ve) from stimulating agent)

1. ChemoTaxis (Chemical)
2. Photo Taxis (Light)

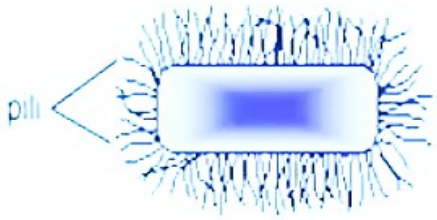
- Axial Filaments (Endoflagella In spirochetes



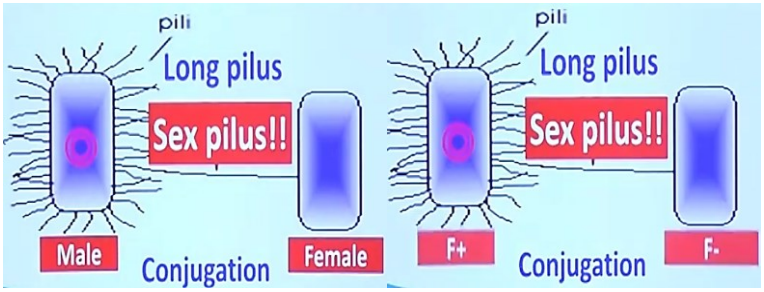


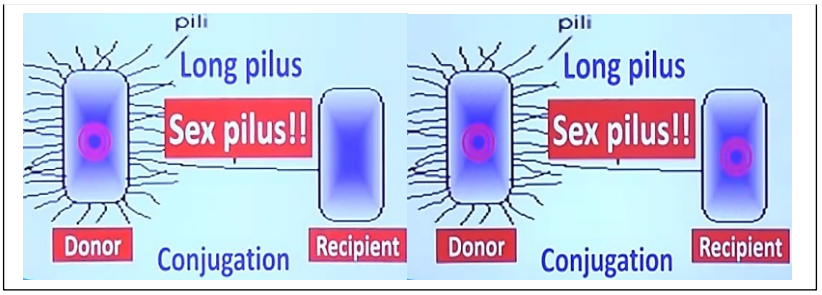


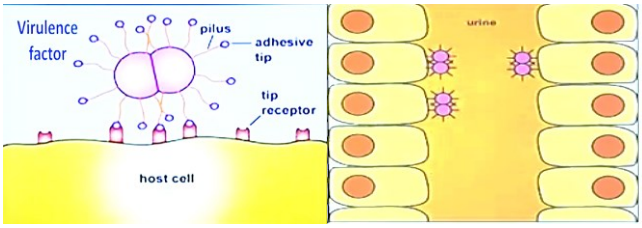
C. Pili (Fimbriae)

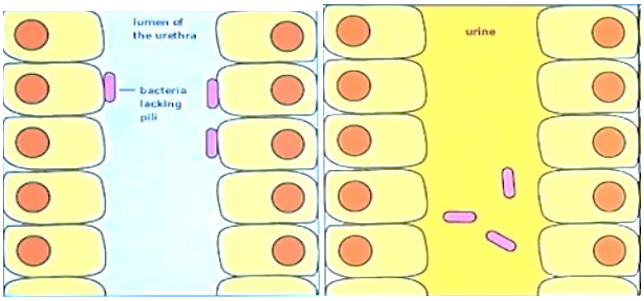


- Short and thin Hair like formed from protein (Pilin)
- Seen by EM (electronmicroscope)
  A) Ordinary pili (Short pili) (Attachment)
  B) Sex pilus (long pili) ((Genetic transfer)









Compare between Pili & Flagella

	Pili	Flagella
1) Structure	Short & Thin	Long & Thick
2) Nature	Protein (Pilin)	Protein (Flagellin) HAg
3) Distribution	Around organism	Mono - Peri
4) Function	1. Attachment 2. Conjugation	Movement (+/-)

Compare between short Pili & sex pilus

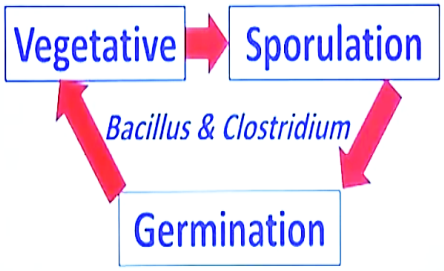
	Short Pili	Sex pilus
1) Arrangement	Numerous	Single
2)Tall	Short	Long
3) Function	Attachment to host cell (Infection)	Attachment to bacterial cell for gene transfer

Q) Why the pili considered as virulence factor?

Attach on host cell and establish the infection

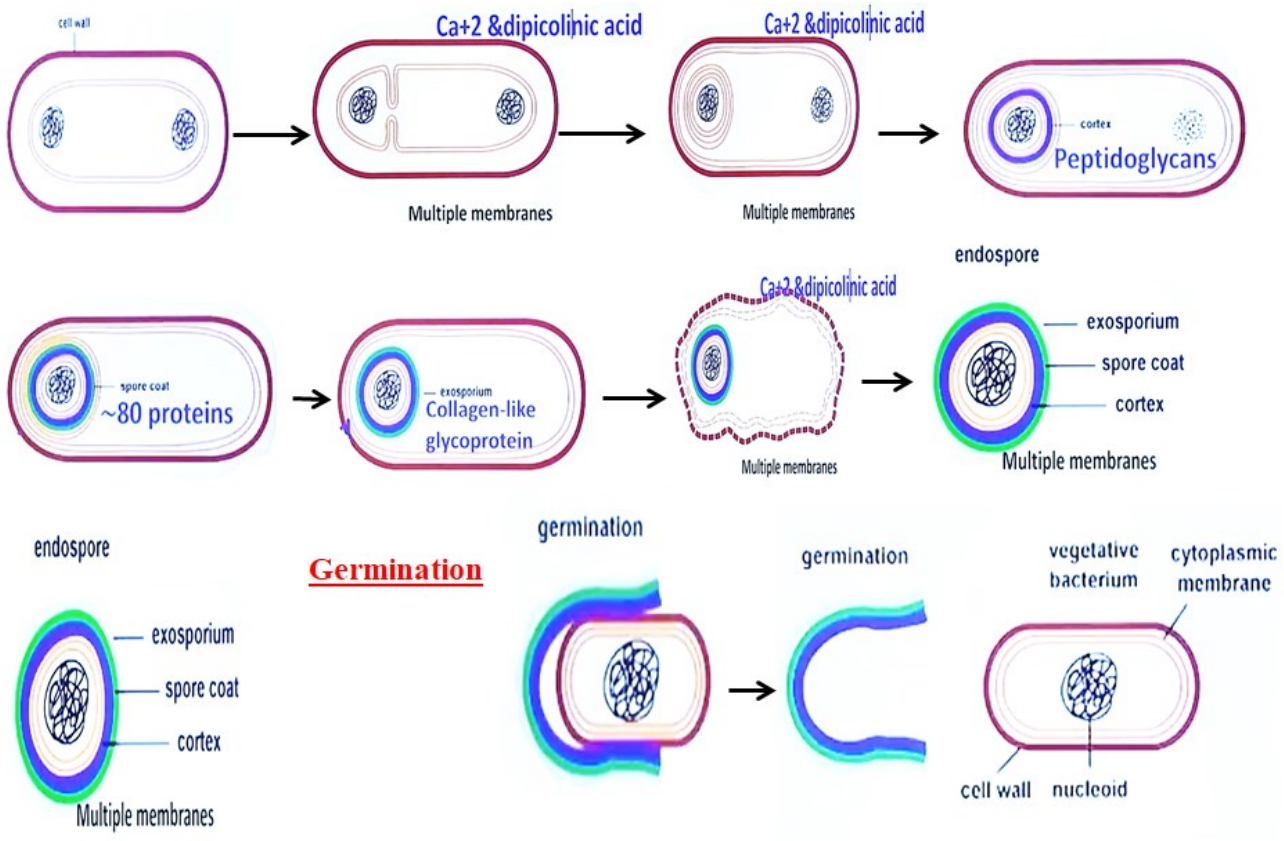
D. Spore formation

1. Vegetative bacteria ⟶ Unsuitable condition ⟶ Spore formation (Outside)
2. Forming highly resistant resting phase (Endospores) in VITRO
   - Bacillus
   - Clostridium
3. Formed outside the body (in VITRO)
4. Cannot stained by ordinary stain
5. Highly resistant to dryness, heat & Disinfectant



Steps spore formation

1. DNA replication
2. Multiple layers **Ca+2 &dipicolinic acid** (Unique to spore)



Position of spores

1. Central & Oval (B. anthracis)	2. Sub-terminal & Oval (Cl. Perfringens)	3. Terminal & Spherical (Cl. Tetani)
		

Harvesting Questions

Q1) Lipid A is a cell wall component of:

A. Gram positive bacteria
B. Fungi
C. Viruses
D. Gram negative bacteria

Q2) One of the following is a function of cell wall

A. Maintain the shape
B. Selective transport
C. Protein synthesis
D. Excretion of extracellular enzymes

Q3) Mycoplasma resist to penicillin due to lack

A. Ribosome
B. Cell wall
C. Cell membrane
D. Pili

Q4) The following is a feature of Gram +ve rather than Gram-ve cell wall

A. Lipid A
B. Outer membrane
C. Thick peptidoglycan
D. Periplasmic space

Q5) Compare between Mycoplasma & L-form bacteria?

Mycoplasma	L-form bacteria
Naturally without cell wall	Induced (cell wall inhibitor)
Cannot re-sensitize cell wall	May sensitize cell wall
Replicate	Cannot Replicate
Survive in any media	Survive isotonic only

Q6) Bacterial capsule:

A) It's organ of movement
B) Makes the organism easily phagocytosed
C) It's an important virulence factor
D) It's usually polypeptide

Q7) One of the following is not about Bacterial spore

A) Spores are resistant to boiling
B) chromosome is not present in the spore
C) Are metabolically inactive
D) Formed only by Bacillus & clostridium

Q8) Structures for attachment

A) Fimbriae (pili)
B) Cell wall
C) Capsule
D) Fimbriae (pili & Capsule)

Q9) Bacteria are protected from phagocytosis by:

A) Capsule
B) Peptidoglycan
C) Mesosomes
D) Flagella

Q10) Choose from the following the essential & nonessential components for bacterial cell?

1. Capsule
2. Plasmid
3. Cell wall
4. Ribosome
5. Flagella
6. Pili
7. Cell membrane
8. Plasmid

Essential	Non-essential
1.	**1.**
2.	**2.**
3.	**3.**
4.	**4.**

Lecture 3

Bacterial Growth and Physiology

- Bacterial reproduction (the process of binary fission)
- Bacterial growth requirements

1. Nutrients
2. Oxygen (O_2)
3. Carbon dioxide (Co_2)
4. Temperature
5. Hydrogen ion concentration (pH)

- **Growth Phases (Bacterial Growth Curve)**

1. **Lag phase**
2. **Log phase**
3. **Stationary phase**
4. **Decline phase**

Bacterial Reproduction

- Bacterial multiplication takes place by simple binary fission

1. The cell grows in size, usually **elongates**

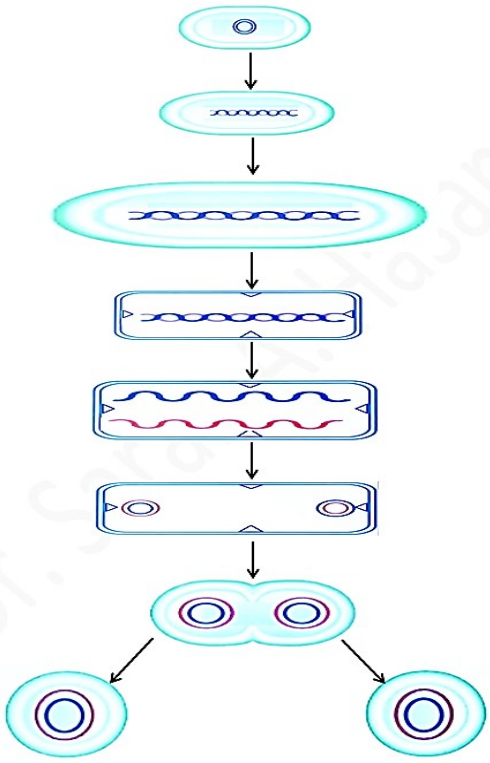
2. The bacterial chromosome acts as **a template** for the replication of **another copy** (Separate ssDNA and become dsDNA)

3. Each copy becomes attached to a **mesosome** on the cytoplasmic membrane.

4. Cell separation

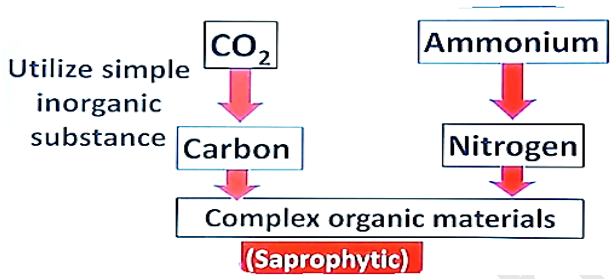
- In some species

1. parent cell completely into two separate daughter cells
2. In others, the cell walls of the daughter cells remain continuous for some time after division giving the characteristic arrangement, e.g. pairs, clusters or chains



Bacterial growth requirements:- In order to grow and divide

1. **Nutrients:** the means by which a particular organism obtains energy, bacteria are classified into: **a-** Autotrophs **b-** Heterotrophs

Autotrophic	Heterotrophic
▪ auto = self ▪ Trophic=nutrition	▪ hetero = different ▪ Trophic = nutrition
▪ These bacteria utilize simple inorganic materials e.g. CO_2 as a source of carbon and ammonium salts as a source of nitrogen	▪ These bacteria require complex organic materials e.g. sugars, proteins etc,
▪ No medical importance (saprophytic)	▪ Medical important (disease)
	

Q/ Why the most pathogenic bacteria are heterotrophic?

It requires organic substance from living host because cannot utilize simple inorganic

2. Oxygen (O_2) : According to O_2 requirements, bacteria are classified into 5 groups:

a- Strict or obligate aerobes (Aerobic respiration) (O_2): require oxygen for growth, e.g. *Pseudomonas aeruginosa.*

b- Strict or obligate anaerobes(Anaerobic respiration) (No O_2): require complete absence of oxygen, e.g. *Bacteroides fragilis.*

c- Facultative anaerobes: generally grow better in presence of oxygen but still are able to grow in its absence, e.g. staphylococci, *E. coli,* ...etc.

d- Micro-aerophilic: organisms require reduced oxygen level, e.g. *Campylobacter* and *Helicobacter.*

e- Aero-tolerant anaerobes have an anaerobic pattern of metabolism but can tolerate the presence of oxygen because they possess superoxide dismutase e.g. *Clostridium perfringens.*

Respiration and energy production: (cellular respiration = glucose catabolism)

- Respiration:- meaning glucose catabolism for energy production
- in **presence** of oxygen, it is called aerobic cellular respiration
- in **absence** of oxygen, it is called anaerobic cellular respiration

Aerobic cellular respiration

- The glucose catabolism under aerobic conditions results in the production of energy in the form of 38 ATP molecules
- superoxide and hydrogen peroxide (H_2O_2) are formed (**highly toxic**)
- aerobic organisms have developed two enzymes, superoxide dismutase and catalase, which detoxify these molecules

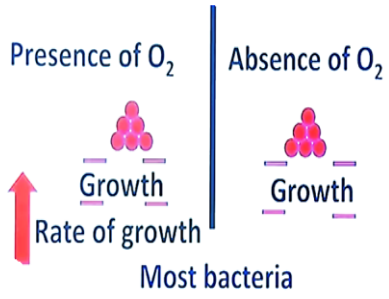
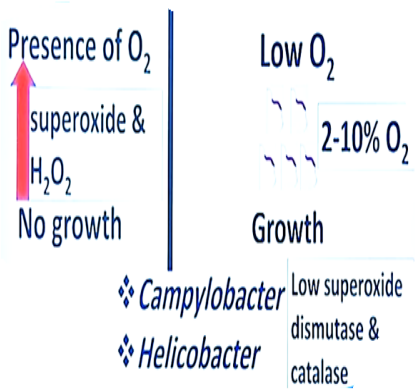
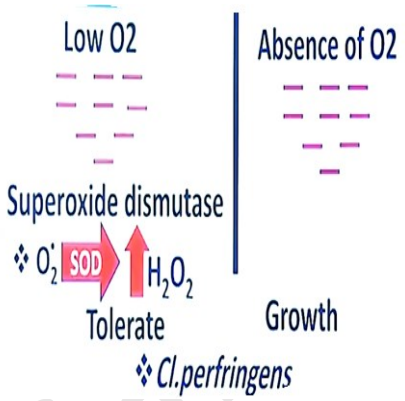
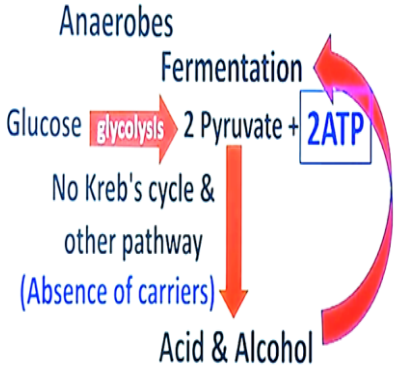
Anaerobic cellular respiration

- It occurs in the **absence** of oxygen
- The net yield of ATP molecules is **less than** it is with aerobic cellular respiration because nitrate, sulfate, and CO_2 are not as good at accepting electrons as oxygen
- obligate anaerobes lack **superoxide dismutase** and **catalase** and so they **cannot grow** in presence of O_2

Fermentation

- It is an anaerobic process (takes place in the absence of oxygen)
- It is used by facultative anaerobes when they exist in an environment that does not contain a nitrate, sulfate, and CO_2
- This is the least efficient method of generating energy

Obligate aerobes (Aerobic respiration)	Obligate anaerobes (Anaerobic respiration)
Presence of O_2 Only Growth | Absence of O_2 No growth *e.g. Pseudomonas aeruginosa*	Presence of O2 No growth | Absence of O2 Only Growth *Bacteroides fragilis*
Aerobic respiration For production Energy (ATP) Glucose catabolism (glycolysis)	-
Glucose +ADP → (2NAD⁺, 2NADH) 2 pyruvate + 2 ATP 2 pyruvate → (Kreb's Cycle) $2FADH_2$ +8NADH + 2 ATP Oxidative phosphorylation O_2 → Carry $2H^+$ → H_2O + 34 ATP 38 ATP	Glucose +ADP → (2NAD⁺, 2NADH) 2 pyruvate + 2 ATP 2 pyruvate → (Kreb's Cycle) $2FADH_2$ +8NADH + 2 ATP 4 ATP ✘ O_2 → Other pathway The organism used inorganic molecules ❖ Nitrate ❖ Sulfate ❖ Co2 Carry H^+ → 13 ATP + 4 17 ATP
Highly toxic molecules Superoxide (O_2^-) (H_2O_2) Superoxide dismutase Catalase	Lack Superoxide dismutase Catalase

Facultative anaerobes	Micro-aerophilic	Aero-tolerant anaerobes
		
		

Q/ Compare between obligate aerobes & obligate anaerobes

Obligate aerobes	Obligate anaerobes
More ATP	Less ATP
Use O_2	Use inorganic nitrate, sulfate, Co_2
Grow only on presence of O_2	Grow only on absence of O_2
Releases toxic Superoxide O_2 , H_2O_2	▪ No Superoxide O_2 ▪ No H_2O_2
Developed ▪ Superoxide dismutase ▪ catalase	Lack ▪ superoxide dismutase ▪ catalase

Q/ Why obligate anaerobes bacteria die in the presence of O_2?

O_2 ⟶ superoxide and H_2O_2

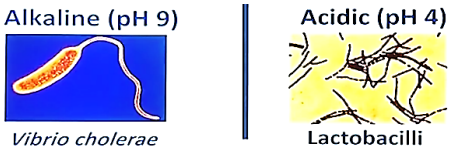
No superoxide dismutase & catalase **(Bacteria will die)**

3. Carbon dioxide (Co_2): The minute amount of Co_2 (0.03%) present in air is sufficient for most bacteria. However, certain species require higher concentrations (5–10%) of Co_2 for growth (capnophilic) e.g. *Neisseria spp.* and *Brucella abortus*

4. Temperature:

- Mesophiles are organisms able to grow within a temperature range of 20–40°C
- Pathogens which replicate on or in human body are able to grow within this range, with an optimum temperature of 37°C which is the normal body temperature
- Psychrophiles (cold-loving) are capable of growth at refrigeration temperature (0- 80C), e.g. *Flavobacterium spp.*
- Thermophiles (heat-loving) grow best at high temperature (>60°C), e.g. *Bacillus stearothermophilus.*

5. Hydrogen ion concentration (pH): Most microorganisms of clinical significance grow best in media whose pH is close to that of human body (pH 7.2). However, some microorganisms grow better at an alkaline pH (8-9), such as *V. cholerae.* Others, such as lactobacilli, prefer media of acidic pH (4 or less).



Growth Phases (Bacterial Growth Curve)

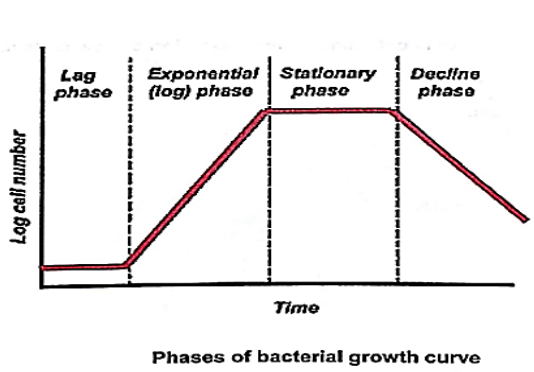
If a small number of an organism is placed in a suitable fluid nutrient medium under appropriate physical and chemical conditions, then the number of viable cells per millilitre is determined periodically, and plotted, a characteristic growth curve with four phases is obtained

1. Lag phase: The initial number of bacterial cells remains constant. During this period, the cells adapt to their new environment. Enzymes and intermediates are formed to permit growth.

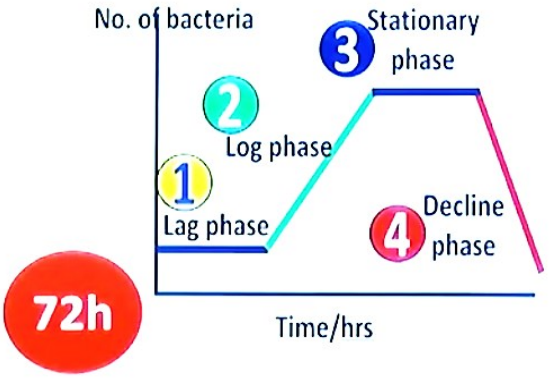
2. Exponential (logarithmic) phase: There is marked increase in cell number. In this phase, the organism shows typical morphology.

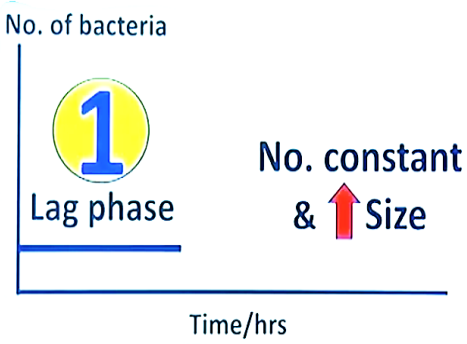
3. Stationary phase: Exhaustion of nutrients and accumulation of toxic products cause growth to decrease. The number of viable bacteria remains constant (balanced).

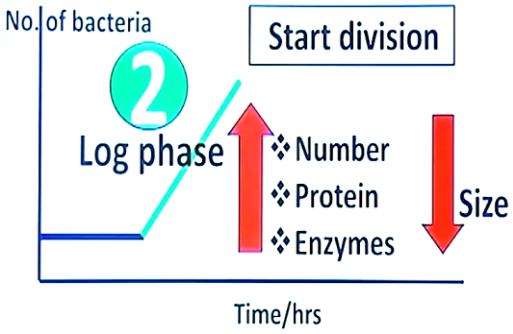
4. Decline phase: At the end of the stationary phase, the death rate increases and exceeds the multiplication rate due to nutrient exhaustion and accumulation of toxic metabolic end products. So, the number of viable bacteria decreases.

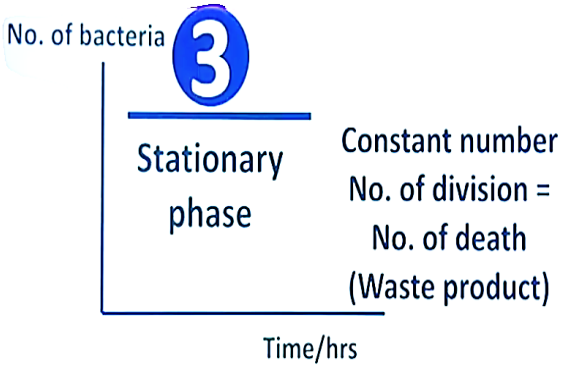


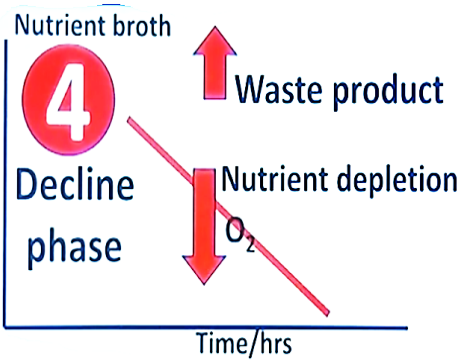
Phases of bacterial growth curve

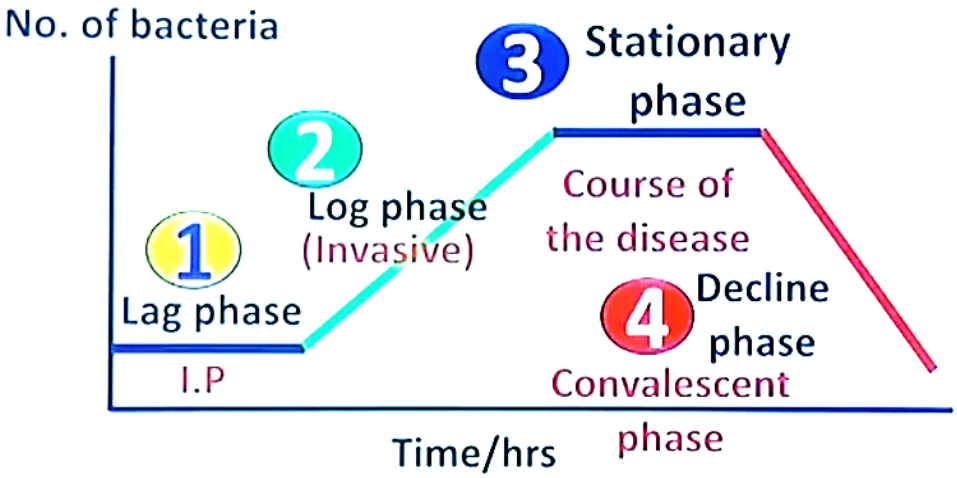












Q) In decline phase no. of death is more than no. of division until no division?

Due to decrease nutrition, O_2 and change in pH so bacteria will die

Q) Number of bacteria in stationary phase is constant?

Balance between division and death

Q) Number of bacteria in lag phase is constant?

Lag phase = no division but bacteria start to adapt itself to the new media (synthesis protein and enzymes for division)

Q) Compare between lag phase and stationary phase?

lag phase	stationary phase
Constant (no division)	Constant (division=death)
Fresh media	Not fresh media
No waste	Accumulation of waste

Harvesting Questions

Q1) What is the type of bacteria that Synthesis organic compounds from nonorganic compounds?

a) Hetrotrophic
b) Obligate anaerob
c) Aerobes
d) Facultative anaerobes
e) Autotrophic

Q2) What is the following term best describes bacteria that lack superoxide dismutase and catalase?

a) Obligate aerobes
b) Obligate anaerob
c) Aero-tolerant anaerobes
d) Faculataive anaerobes
e) Microaerohhilcs

Q3) What is the following term best describes bacteria that lack catalase but not superoxide dismutase?

a) Obligate aerobes
b) Obligate anaerobes
c) Facultative anaerobes
d) Micro-aerophilc
e) Aero-tolerant anaerobes

Q4) Cabnophinic bacteria required

a) Low concentration of O_2
b) High concentration of O_2
c) Low concentration of CO_2
d) High concentration of CO_2
e) High alkaline of pH

Q5) What type of bacterium is most likely to cause spoilage of refrigerated foods?

a) Mesophilic
b) Thermopjilic
c) Psychrophilic
d) Capnophilic
e) Microaerophilic

Lecture 4

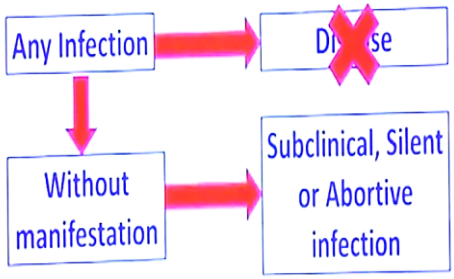
Bacterial Pathogenesis

1. Relation between bacteria and host
2. Stages of infectious process
3. Koch's postulate
4. Microbial virulence
   - Definitions
   - Virulence Factors of Bacteria

A. Adherence factors
B. Invasion factors
C. Toxin production

Infection is a process by which the organism enters into a relationship with the host.

- clinical disease (with symptom)
- subclinical, silent or abortive infections (without symptom)

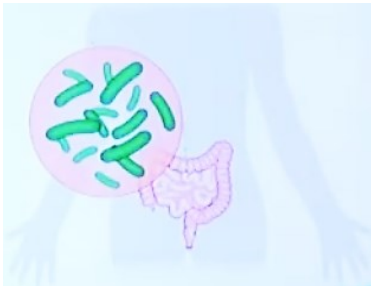


- The relationship between bacteria and host could be classified into

1. Saprophytic bacteria: are those which live freely in nature, on decaying organic matter, in soil or water. **They do not require a living host**

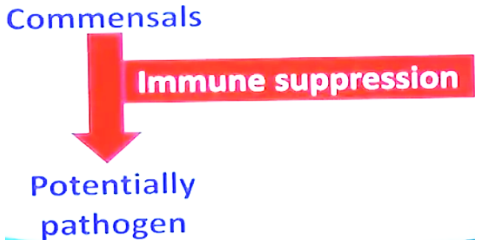
2. Parasitic bacteria: are those which live on or in a living host. They are classified according to their relation to the host into

a- Pathogenic: Bacteria capable of causi



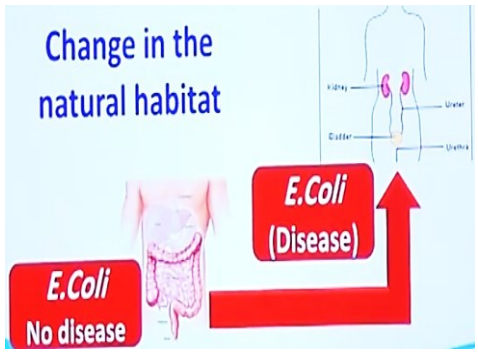
b- Non-pathogenic (commensals)

- Not cause disease (part of the nor
- May be present in
  - ➢ Large number
  - ➢ Scanty
  - ➢ Sterile
- Inhibits pathogenic bacteria
  - ✓ Competition
  - ✓ Bactericidal
  - ✓ Decrease pH

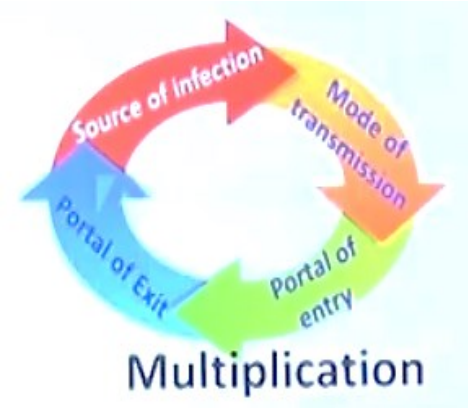


c- Opportunistic pathogens:

- These are potentially pathogenic bacteria (Real pathogen)
- do not cause disease under normal conditions but can cause disease in immunocompromised patients, or when they find their way to another site other than their normal habitat e.g. subacute endocarditis (Viridans steptococci)
- Many of these opportunistic pathogens are **originally commensals**



Stages of the Infectious Process



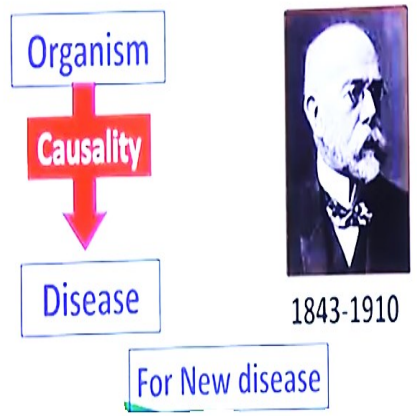
1. Source of infection which may be man (case or carrier), animal or soil

2. Mode of transmission e.g. droplet inhalation, ingestion, injection, insects bite, sexual contact, bite and transplacental

3. Portal of entry e.g. respiratory tract, gastrointestinal tract, genitourinary tract injured skin...etc. **The organism then starts to multiply within the host causing tissue damage (disease)**

4. Portal of exit e.g. urine, stools, blood, respiratory or genital discharge, from which the organism is transmitted to a new host

Koch's postulates



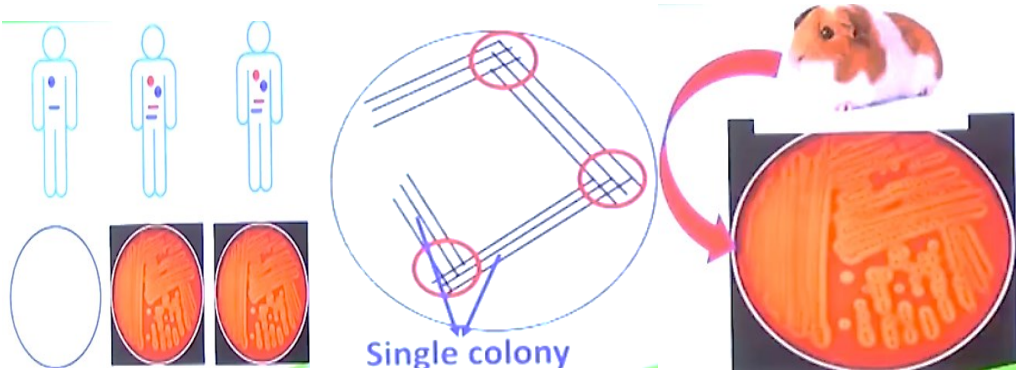
These are criteria that were proposed by Koch In order to determine if the organism isolated from the patient actually caused the disease, these criteria are as follows:

1. The organism must be isolated from every patient with the disease.

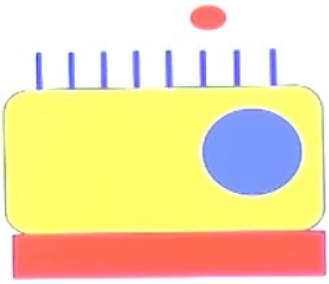
2. The organism must be isolated free from all other organisms and grown in pure culture in vitro.

3. The pure organism must cause the disease in a healthy, susceptible animal.

4. The organism must be re-isolated from the inoculated animal.



Microbial Virulence



- **Pathogenicity:-** it is the ability of an organism to cause disease (Qualitative)
- **Virulence**:- degree of pathogenicity (Quantitative)

Virulence Factors of Bacteria

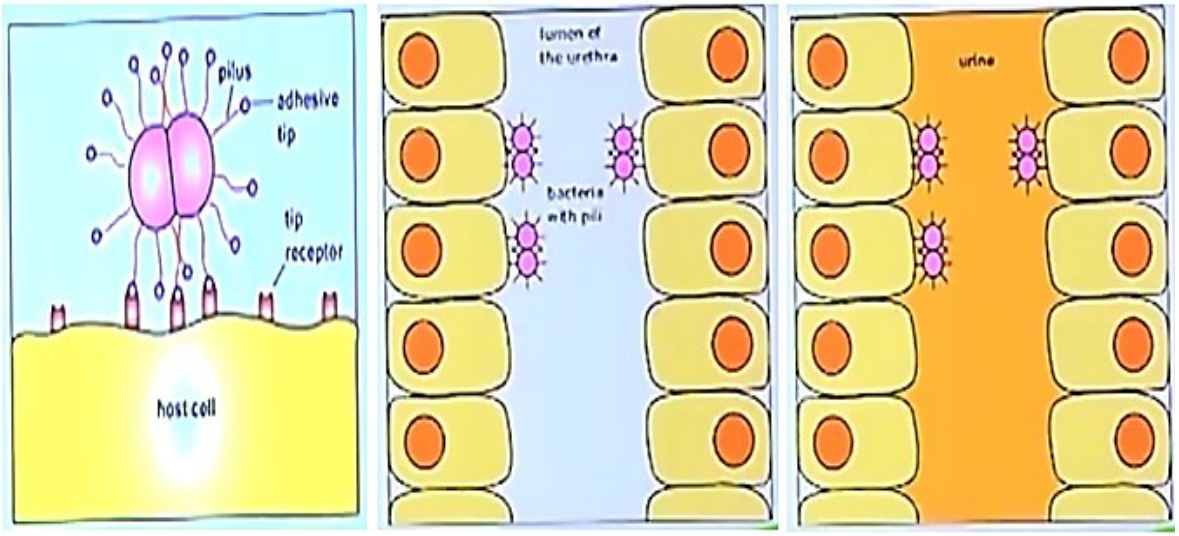
- **A virulence factor:-** is either a structure (e.g. capsule) or a product (e.g. toxins) that enables the organism to cause disease

A- Adherence factors

They enable bacteria to attach to the host surfaces, thus contributing to the establishment of the infection. For example:

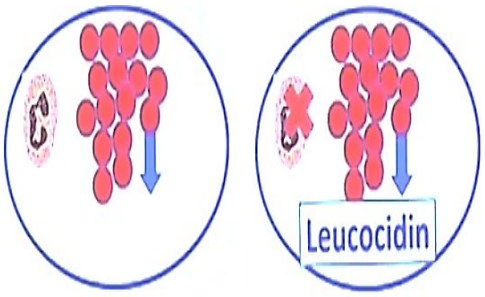
1. The fimbriae (Pilli) of Neisseria gonorrhoeae and E. coli help the attachment of these organisms to the urinary tract epithelium.

2. The glycocalyx of Staphylococcus epidermidis and certain viridans streptococci allows the organisms to adhere strongly to the heart valves
   - Streptococci mutans (Dental caries)



fimbriae (Pilli) (Adherence factors)

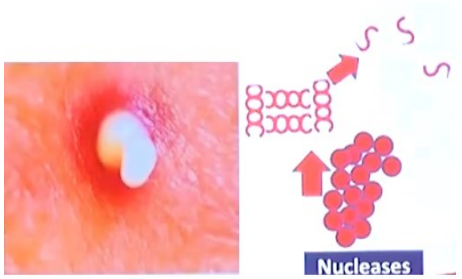
B- Invasion factors



Invasion of tissue followed by inflammation is one of the main mechanisms by which bacteria can cause disease. This invasion is helped by:

1. Enzymes (Degrade host tissue)

a- Collagenase and hyaluronidase which degrade collagen and hyaluronic acid and allow the bacteria to spread through subcutaneous tissues

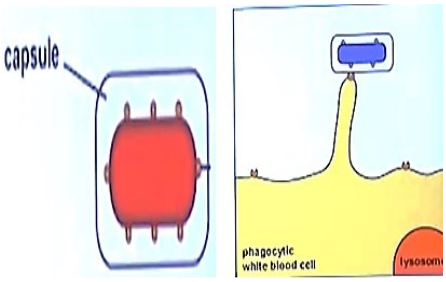


b- Immunoglobulin A protease which degrades IgA

c- Leukocidin which can destroy leucocytes and macrophages inside fibrin

d- Deoxyribonuclease that breaks down DNA

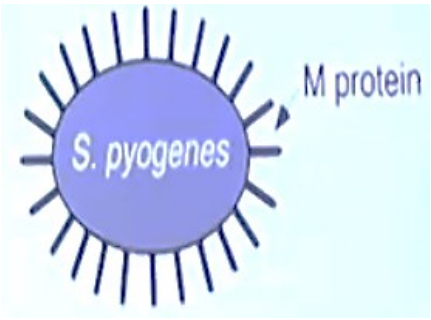
e- Lecithinase that breaks down lecithin of cell membrane



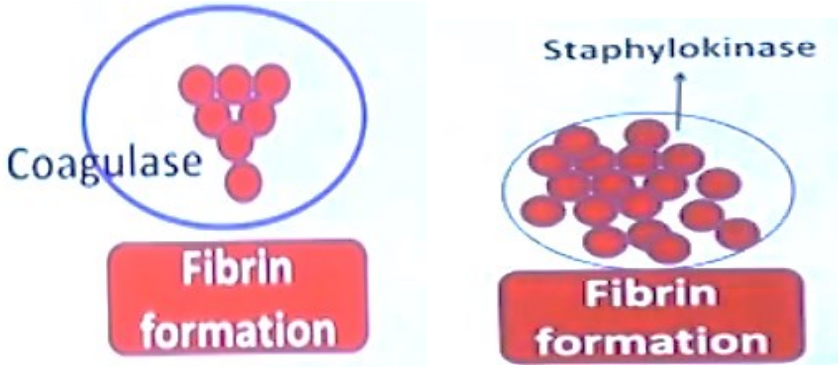
2. Antiphagocytic factors

a- Capsule: The capsule prevents the phagocytes from attachment to the bacteria, e.g. *Strept. pneumoniae.*

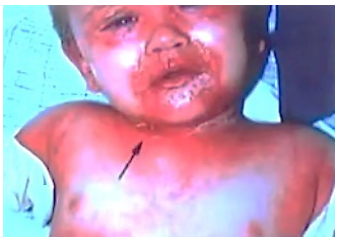
b- Cell wall proteins of Gram-positive cocci, such as the M protein of *Strept. pyogenes* and protein A of *Staph. aureus*



c- Coagulase: It accelerates the formation of a fibrin clot from fibrinogen. This clot can protect bacteria from phagocytosis, e.g. *Staph. aureus.*

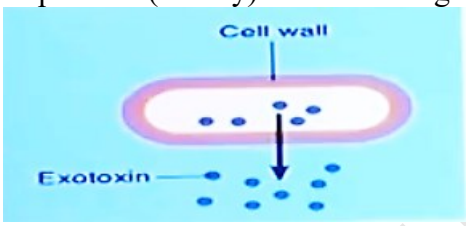
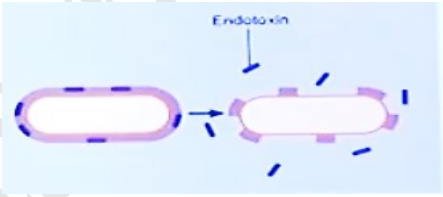
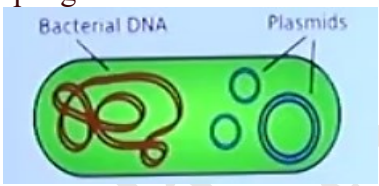
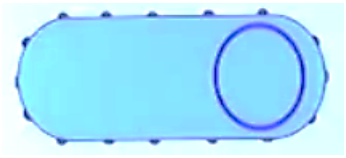
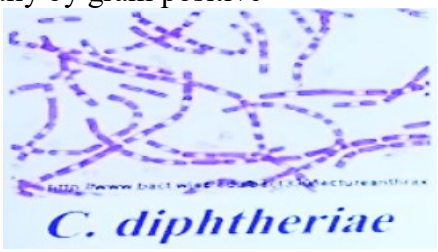


C– Toxin production



Toxin production is another mechanism by which bacteria can **produce disease**. Bacterial toxins are either **exo**toxins or **endo**toxins

Comparison of the main features of exotoxins and endotoxin

	Exotoxins	Endotoxins
Source	Secreted by living bacterial cells both Gram-positive (mainly) and Gram-negative 	▪ Integral part of the cell wall of Gram-negative organisms. ▪ Liberated when the bacteria die 
Coding genes	Encoded by chromosomes, plasmids, bacteriophages 	Encoded by genes on the chromosome only 
Nature	Protein	Lipopolysaccharide (lipid A)
Antigenicity	▪ Highly antigenic(so produce Protective antibodies)	Poorly antigenic (Antibodies not protective)
Heat stability	Unstable to temp. above 60°C	Stable to temp. above 60°C for several hours
Detoxification	Can be converted into toxoid* (Vaccine)	Cannot converted (No vaccine)
Specificity	▪ Every toxin has specific action ▪ NO Fever ▪ different types of toxin according to bacteria	▪ Same generalized effect (non-specific action) ▪ all give fever and shock ▪ Same toxin in all bacteria
Toxicity	High	Low
Type of bacteria	Usually by gram positive 	usually by gram negative 

❖ Treatment of exototoxin with formalin (or other agents) removes its toxicity and retains its antigenicity converting it into **toxoid**, that can be used for immunization

Harvesting Questions

1- Opportunistic pathogens

a) Are never the cause of a clinical infection
b) Are usually highly pathogenic
c) Are rarely part of the normal flora
d) Cause disease mainly in immunocompromised individuals
e) Are resistant to killing by steam sterilization

2- Exotoxins have the following characters, EXCEPT

a) They may be encoded by genes on the chromosome.
b) They can be converted to toxoids.
c) They have specific action.
d) They are polypeptides.
e) They are heat stable.

3- Endotoxlns

a) Are secreted mainly by Gram-positive bacteria
b) Are highly antigenic
c) Are stable at temperatures above 60°C
d) Can be converted into toxoid
e) Have specific action

Lecture5

Disinfection and Sterilization

Define terms: sterilization, disinfection, disinfectant, antiseptics, germicide, cleaning and decontamination

- **methods of disinfection**

A. Physical method

1. Moist heat (pasteurization, boiling)
2. Radiation (UV rays)

B. Chemical method

1. Low level disinfectants
2. Intermediate level disinfectants
3. High level disinfectants

- **methods of sterilization**

A. Physical methods

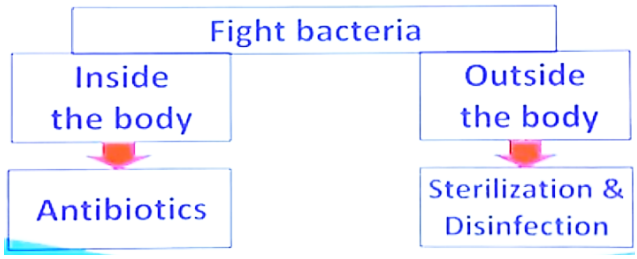
1. Moist heat (Autoclave)
2. Dry heat (incineration, direct flame, hot air oven)
3. Ionizing radiation
4. Filtration

B. Chemical methods

1. Gaseous(H_2O_2, Ethylene oxide, Peracetic acid gas)
2. Liquid

Fight bacteria

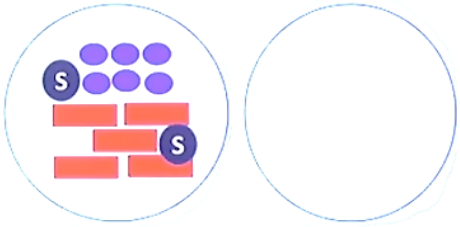
1. Inside the body (Antibiotics)
2. Outside the body (Sterilization and Disinfection)



Definition and principles of terms

Sterilization:

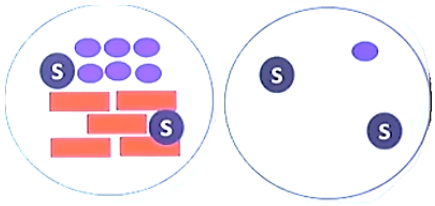
- Removal of all forms of viable microorganisms including all bacterial spores by physical or chemical methods.
- Absolute term:- killing or removing all microorganisms
- Sterilization is essential for
  1. culture media
  2. surgical instruments.
  3. Syringes, Gloves and catheters



Sterilization

Disinfection:

- It is a process that eliminates most, if not all, pathogenic microorganisms except spores by physical or chemical methods.



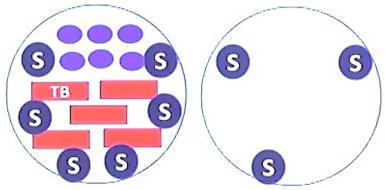
Disinfection

Disinfectant (toxic):

- Usually a chemical agent that achieves disinfection (applied to inanimate objects)
- Disinfectants may be categorized into 3 levels: high, intermediate and low:

1. High level disinfectant:

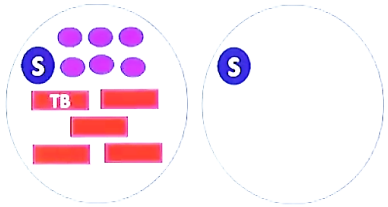
kills all microbial pathogens except large numbers bacterial spores. Examples include hydrogen peroxide for contact lens, chlorine for blood spills.



High level disinfectant

2. Intermediate level disinfectant:

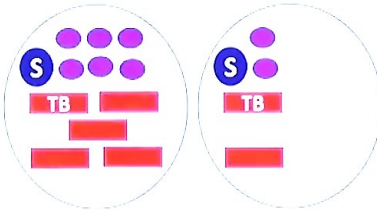
- Kills most (all) Microbes, **EXCEPT** bacterial Spore
- Examples include alcohol.



Intermediate level disinfectant

3. Low level disinfectant:

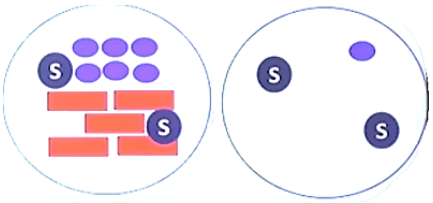
- Kills MOST microbes, **EXCEPT** TB & bacterial Spore
- Example:- quaternary ammonium compounds like
  - Benzethonium Chloride
  - Benzalkonium chloride
- Used for disinfection of floors and blood spills



Low level disinfectant

Antiseptic (non-toxic):

define same as disinfection but can be safely applied to skin and mucous membranes (not suitable for systemic administration).



Disinfection/ antiseptic

Germicide:

- Agent that destroys microorganisms; may be

1. Virucide 2. Bactericide 3. Fungicide 4. sporicide

- The term germicide includes **disinfectant , antiseptic or sterilant**

➢ **Disinfectant:** Chemical germicide that achieves Disinfection (disinfectants applied only to inanimate objects)

➢ **Antiseptic:** Chemical germicide that achieves Disinfection (applied to living tissue and skin)

➢ **Sterilant:** Chemical germicide that achieves sterilization

Cleaning:

- Removal of foreign material from medical devices as part of decontamination process.
- It is usually done with **water and soap**.
- Cleaning must always precede disinfection and sterilization.

Decontamination:

- Reduction of pathogenic microorganisms to a level at which items are safe to handle.
- Decontamination **Include: cleaning, disinfection or sterilization**

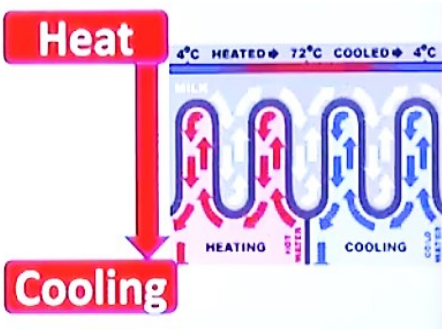
Q) Compare between Sterilization & disinfection?

Sterilization	Disinfection
Kill all organism	kill most organism
Kill spore forming	Can not
Physical or chemical	Physical or chemical

Main methods of disinfection

A. Physical methods for disinfection:- include

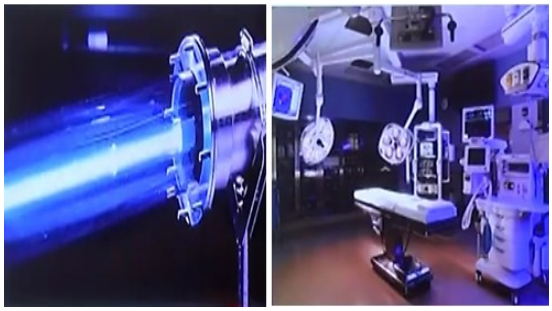
I. Moist Heat



Pasteurization

1. **Pasteurization:** Moist heat below 100°C, pasteurization of milk by heating at 63°C for 30 min. or at 72°C for 20 sec., followed by rapid cooling, destroys important pathogens e.g. Mycobacterium tuberculosis, Brucella, Salmonella and Coxiella bumetti.
2. **Boiling:** Moist heat at 100°C for 20 min. achieves high disinfection (kill all vegetative bacteria). It can be useful in emergencies if sterilizer is not available (glass syringes, surgical instruments).

II. Radiation (Ultraviolet rays) (UV)


UV rays

- UV rays have weak penetration power
- regarded as
  1. Bactericidal
  2. Carcinogen
- air and surface disinfection, e.g.,
  1. Operation room
  2. Drug filling cubicles
  3. laboratory safety cabinets

Q) Why UV rays used for disinfection but not for sterilization

Because UV rays are low penetration power

B. Chemical methods for disinfection:- include

1. Low level disinfectants 2. Intermediate level disinfectants 3. High level disinfectants

Intermediate level disinfection

1. Alcohols (Alcohol 70%)

- such as:-
  - Ethanol (Ethyl alcohol)
  - Isopropanol (Isopropyl alcohol) (More stable)
- Act as:-
  - Bactericidal
  - Fungicidal
  - Viricidal (Enveloped)
- Kill microbes by:-
  1. Denaturation
  2. Membrane damage
  3. Disruption of lipid containing
  4. Used as:- Antiseptics (Hand sanitizers)
- **Knots:-** Methanol (Methyl alcohol) causing
  - Blindness
  - Damage in brain (Death)

Q) Which solution of ethyl alcohol is more effective at inhibiting microbial growth: a 70% solution or a 100% solution?

4. Halogens

- includes

A. Chlorines (High level disinfectant)

B. Iodines

1. Iodines Tincture Iodine (Skin antiseptics)
   - (2% Iodine + 2.4% sodium iodide in 50% ethanol)
2. Betadine (Povidone + Iodine)
   - (More stable)

C. Fluorine (Toothpaste)

- **Halogens** Kill microbes by
  1. Oxidation
  2. Denaturation

2. Phenols

- First used in the operation room by Lister in 1867.
- Phenol derivatives (Cresol (Lysol), Chloroxylenol)
- kill microbes by:-

1. Denaturation
2. Membrane damage

- Disinfectants (floors, culture spills)

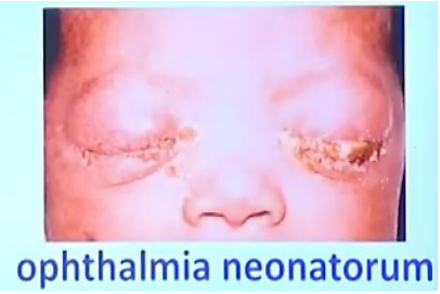
3. Chlorhexidine (Biguanides)

- Antiseptic (Mouth washing)

5. Heavy metals (Antimicrobial activity)

- Includes (Copper, Nickle and Zinc)
- kill microbes by:-
  1. Denaturation
  2. Inhibition enzymatic activity
- Toxic to human & animal in excessive concentration (Argyria)
- Copper, Nickle and Zinc, used in made doorknobs in hospital
- Sliver
  - Drinking water was stored in silver jugs)
  - Silver nitrate drops (ophthalmia neonatorum)
- Zinic (Zinic oxide)
  - Calamine lotion
  - Baby powder

C. High level disinfectant



1. **Chlorine (halogen)**
   - Disinfectant of water and swimming pool
2. **Sodium Hypochlorite (Chlorine+ Sodium + Oxygen)**
   - Disinfectant in homes & hospitals
3. **Hydrogen peroxide (Antiseptic)**
4. **Glutaraldehyde 2% and Peracetic acid**
   - Needs 10 hours

Q) Hydrogen peroxidase is better used for antiseptic against obligate anaerobic bacteria than obligate aerobes? Why?

Q) Why there is resistant to antibiotics and usually no resistant for chemical disinfectants?

Because Chemical disinfectants have a combination action includes:-

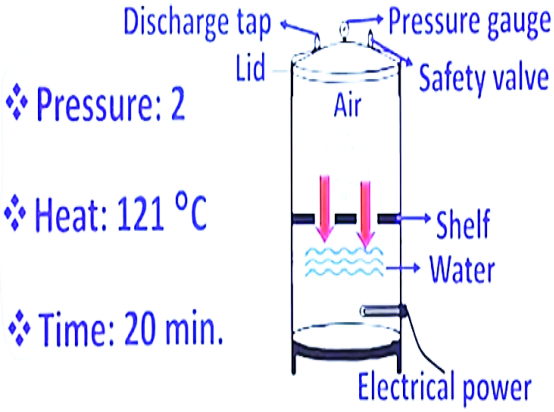
1. Oxidation
2. Denaturation
3. Breaks DNA
4. Cell membrane and cell wall damage

Main methods of sterilization

A. Physical methods for sterilization

1. Steam sterilization (Moist heat above 100°C) (autoclave)

- There are four parameters of steam sterilization:
  1. Saturated steam
  2. Pressure
  3. Temperature
  4. time
- The two common steam-sterilizing temperatures are
1. 121°C for 20-30 minutes under 2 pressure
2. 132°C for 4 minutes under 3 pressure
- mode of action:- moist heat destroys microorganisms by coagulation and denaturation of enzymes and structural proteins.
- Used for sterilization of:
  1. Surgical instruments
  2. Bed linen
  3. Surgical dressings
  4. Gauze
  5. Cotton



- **Advantages**
  1. Nontoxic, inexpensive
  2. rapidly heats
  3. High penetration
  4. Rapid
- **Disadvantages**
  1. Not suitable corrosion-susceptible metal
  2. Sterilized objects moist

Monitoring of steam sterilizers (autoclaves), use following 3 monitors:

1. Mechanical indicators:

2. Chemical indicators or integrators: Chemically impregnated paper strips (Visible colour changes)

3. Biological indicators:

- Paper strips impregnated with the spores of Geobacillus stearothermophilus.
- spore strips are incubated in a fluid medium at 37°C for 48h. Absence of bacterial growth indicates an efficient sterilization cycle.

Q) Why the air must be removed from the autoclave system? Because the air act as a barrier and lead to block penetration of steam

2. Dry heat sterilization

includes the following forms:

1. Incineration

- applicable for dead animal bodies
- infectious hospital waste such as used surgical dressings, needles....etc.

2. Red heat (Direct flame)

- loops and points of forceps

3. hot air ovens

- temperature

1. 170°C for 60 minutes
2. 160°C for 120 minutes
3. 150°C for 150 minutes.

- Mode of action, killing is due to oxidation of the microbial cell constituents
- used for materials that might be damaged by moist heat (e.g., powders and sharp instruments).
- The advantages

1. Nontoxic
2. Inexpensive
3. noncorrosive for metal and sharp instruments

- The disadvantages

1. slow rate of heat penetration
2. time-consuming
3. the high temperatures are not suitable for most materials

Q) Compare between Hot air oven & Autoclave

Property	Dry heat	Moist heat
1- Mechanism of action	By oxidation	By denaturation
2- Effective temp.	High (160 or 170°C)	Low (121 C)
3- Time	1 or 2 hours	20 minute
4- Penetration rate	NO	Yes
5- Latent heat	NO	Yes
6- Sterilized objects	Dry	Moist

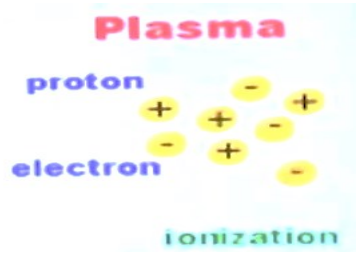
3. Ionizing radiation

- Sterilization by gamma rays or βeta rays
- a high penetrating power (Breaks DNA)
- used for sterilization of prepacked heat-sensitive items such as bone grafts, surgical sutures, disposable plastic syringes, gloves, catheters and plastic Petri dishes.

4. Filtration

- fluids sterilization (antibiotic solutions, hormones, vitamins) that cannot be purified by any other means.
- Fluid filters with pore size as small as 0.22 μm.
- Air filtration (operating rooms, drug factories and laboratory biosafety cabinets)

B. Chemical methods for sterilization



- Gaseous

1. Hydrogen peroxide gas plasma

- Gas plasmas:- the fourth state of matter (i.e., liquids, solids, gases, and gas plasmas).
- Plasma = any gas that contains electrons, ions
- using radio frequency or microwave energy to excite the gas molecules (free radicals)
- mode of action:- The free radicals interact with essential cell components (e.g., enzymes, nucleic acids) and thereby disrupt the metabolism of microorganisms
- Total time of sterilization cycle is about 50 minutes.

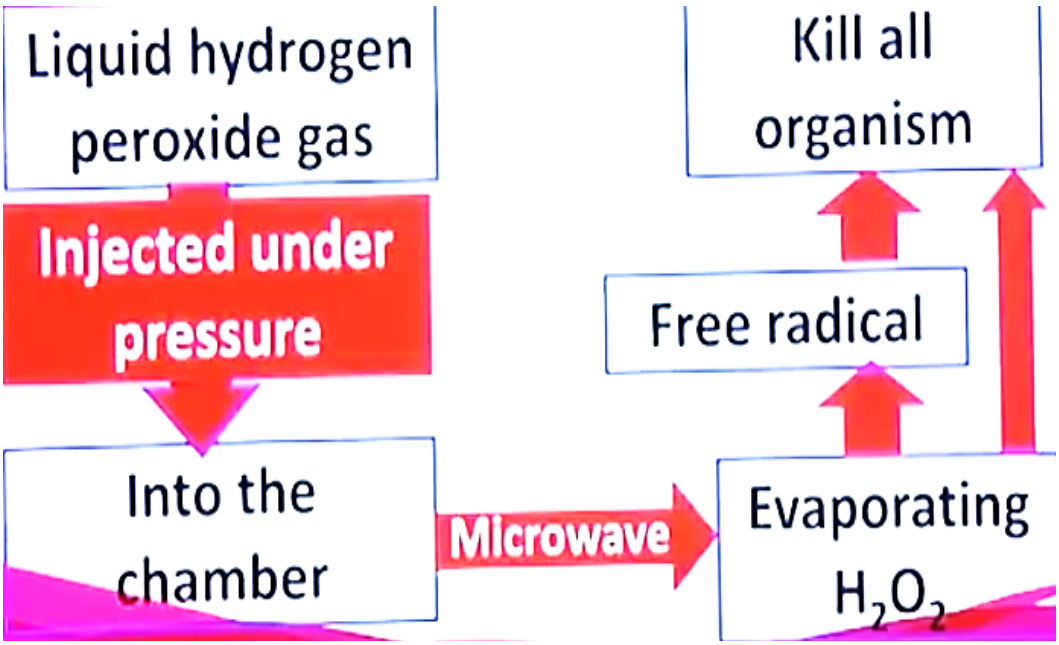
- **It is used to sterilize**

1. Heat sensitive devices (plastics)
2. electrical devices
3. corrosion-susceptible metal

- **Advantages**

1. Non-toxic
2. Suitable for Heat sensitive

- **Disadvantages** include total time of sterilization cycle is about 50 minutes



2. Ethylene oxide gas sterilization (EO)

- Kill all organism including spores
- It can be used for instruments that cannot be subjected to steam.
- disadvantage

1. Exposure time (3 to 6 hours)
2. expensive with probable toxicity
3. Then aerated for 8–12 hr. to remove any trace of the gas

3. Peracetic acid sterilization (The best sterilant)

- Acetic acid and hydrogen peroxide
- It is used to sterilize medical, surgical, and dental instruments (e.g., endoscopes, arthroscopes).
- mode of action:–

1. denatures proteins
2. disrupts cell wall, and oxidizes proteins and enzymes of microbes.

- Liquids (Chemical solution)

1) Glutaraldehyde 2%
   - High level disinfectant for 20 min.
   - Sterilization (10hrs)
2) Peracetic acid

Q) by using the heat, classify the sterilization and disinfection?

Sterilization		Disinfection (moist heat)
Dry heat	**Moist heat**	a) Below 100 °C b) At 100 °C
a) Incineration b) Direct flame c) Hot air oven	Above 100 °C (the autoclave)	

Q) by using the chemical substances, classify the sterilization and disinfection?

Chemical sterilization		Chemical disinfection
Gaseous	**Liquid**	1. Low level disinfectants 2. Intermediate level disinfectants 3. High level disinfectants
a) Hydrogen peroxide Plasma gas b) Ethylene oxide gas c) Peracetic acid gas	a) Glutaraldehyde b) Peracetic acid	

Harvesting Questions

Q1) A Chemical substance that kills most pathogenic organisms but does not kill spores

A. Sterilant
B. Disinfection
C. Disinfectant
D. Cleaning

Q2) Antiseptics like disinfectants for living tissues

- False
- True

Q3)Doorknobs and other surfaces in clinical settings are often coated with_____, _____, or _____to prevent the transmission of microbes.

Q4) Bleach is an example of which group of chemicals used for disinfection?

A. Heavy metals
B. Halogens
C. Alcohols
D. Phenols

Q5) One of the following statements is CORRECT:

A. Sterilization is complete removal or inactivation of all forms of microbial life.
B. Disinfection is elimination of all pathogenic organisms including spores.
C. Low level disinfection is effective against Mycobacterium tuberculosis.
D. Antiseptics are chemical disinfectants applied to surfaces and floors.
E. High level disinfection is enough for surgical instruments and needles.

Lecture 6

Streptococcus

Characters of the genus Streptococcus

1. Gram-positive ovoid cocci
2. Arranged in chains or pairs
3. Catalase negative: Catalase test is a key test for discriminating streptococci from the catalase-positive staphylococci (Staph (+) x Strept (-)).
4. Growth requires enriched media containing blood or serum.

Most important species

1. streptococcus pyogenes
2. Streptococcus agalactiae
3. Streptococcus pneumonia

- The medically significant streptococci may be conveniently divided on the basis of:

1. Haemolysis on blood agar
   - Complete haemolysis, beta
   - Partial haemolysis, alpha
   - No haemolysis
2. A group-specific carbohydrate antigen, according to which streptococci are classified into groups (A-H) to (K-U) (Lancefield classification)

Beta-Haemolytic Streptococci

1-Streptococcus pyogenes (Group A Streptococcus):

Morphology

S. pyogenes are Gram positive cocci in chains.

Cultural characters

- S. pyogenes produce beta-haemolysis on blood agar.
- S. pyogenes growth is inhibited by bacitracin.

Virulence factors and pathogenesis

A. Factors that mediate adherence (colonization):

1. M protein (most important virulent factor)
   - Adherence, anti-phagocytosis
   - Major surface antigen (immunogenic)
   - divides S. pyogenes into about 90 serotypes
2. Lipoteichoic acids
3. Fibronectin-binding protein (F Protein)

B. Factors that mediate invasion:

1. Antiphagocytic factors:

a- M protein.

b- C5a peptidase (breaks down C5a so that it no longer attracts phagocytes)(chemotactic)

c- Hyaluronic acid capsule:

- It is chemically similar to that of host connective tissue
- not immunogenic

2. Invasins:

a- Streptokinase (fibrinolysin):

- used for emergency therapy of myocardial infarction to remove blood clots

b- Streptolysins (haemolysins): These are two pore-forming toxins that lyse host cell membranes.

1. Streptolysin o (oxygen labile) is a highly immunogenic protein
2. Streptolysin S (oxygen stable) is non-immunogenic

c- Streptococcal pyrogenic exotoxins (SPE- A, B & C): These toxins act as superantigens causing toxic shock syndrome, septicaemia, In addition:

- Toxin A is an erythrogenic toxin (red rash characteristic of scarlet fever).
- Toxin B acts as a protease. It contributes to the pathogenesis of necrotizing fasciitis.

d- Others: e.g., hyaluronidase and nucleases(DNAase). These enzymes, together with streptokinase, contribute to the spreading nature of streptococcal infections.

Streptococcus pyogenes Infections

I. Localized infections:

1. Pharyngitis (sore throat, tonsillitis)

- It is the **commonest** infection
- It is characterized by pain, redness and swelling of posterior pharynx, accompanied by greyish white tonsillar exudate and fever.

2. Scarlet fever

- erythrogenic toxin
- It is characterized by development of scarlet red rash
- strawberry tongue

3. Skin and soft tissue infections

- Impetigo ⟶ blisters ⟶ pus
- Cellulitis ⟶ deep layers of the skin
- Erysipelas: It is a form of cellulitis accompanied by fever and systemic toxicity

II. Invasive infections:

1. **Puerperal fever:** delivery or abortion
2. **Acute endocarditis:** normal or damaged heart valves
3. **Necrotizing fasciitis:**
   - severe tissue destruction particularly associated with SPE-B (protease).
   - flesh-eating bacteria
4. **Toxic shock syndrome:**
   - This condition is mediated by the production of SPE-A, B & C
   - It often begins with skin wounds or minor traumas
   - High fever, hypotension, skin rash, renal failure and multi-organ failure

Laboratory diagnosis

A. Specimens include throat swab, pus, blood in invasive infections

B. Direct detection

1- **Gram-stained smears** are useful only in cases of skin and soft tissue infections since S. pyogenes cannot be visually distinguished from the normal oral streptococcal flora

2- **Detection of Lancefield group A streptococcal antigen** in throat swab (specific antibody for CHO Ag)

C. Cultivation

- blood agar
- blood culture technique (Blood samples)

D. Identification

After 24h incubation, the growth should be examined

1. Catalase test: Staph (+) x Strept (-)
2. On blood agar: beta-haemolysis
3. Gram-stained film: (colony, culture)
   - Gram positive, ovoid cocci
   - Arranged in chains
4. S. pyogenes can further be identified by:
   - Growth inhibition by bacitracin (sensitive)
   - Agglutination by group A antibody (colony, culture)

Post-Streptococcal Sequelae

(Acute Rheumatic Fever and Acute Glomerulonephritis)

Comparison between acute rheumatic fever (ARF) & acute glomerulonephritis (AGN)

	ARF	AGN
1. **Age**	Any age (mostly 4-30 years)	Children more than adults
2. **Pathogenesis**	▪ Anti-M protein antibodies ▪ Cross-reaction ▪ Damage heart (carditis) ▪ autoimmune reaction ▪ by recurrence of infection leading to valvular damage ▪ 2-3 % normal population	▪ Zone phenomenon ▪ Ag = Ab (immune complex deposition) ▪ More Ag = Less Ab ▪ Low Ag = More Ab ▪ soluble immune complex
3. **S. pyogenes strains**	Rheumatogenic (1, 5, 6, 3, 18)	Nephritogenic (4, 25, 2, 60)
4. **Precipitating infection**	Pharyngitis (but not skin infection)	Pharyngitis and Skin infections
5. **Chemoprophylaxis**	▪ Essential ▪ **Long acting penicillin** is recommended following a single attack ▪ **Erythromycin** (penicillin-allergic patients)	Unnecessary
6. **Sequelae**	Heart disease	Rarely, chronic renal failure
7. **Early treatment of precipitating Inf.**	Prevent the condition	Does not prevents the condition

2. Streptococcus agalactiae (Group B Streptococcus; GBS)

- Group B
- beta-haemolytic
- Bacitracin resistant
- polysaccharide capsule (virulent factor, anti-phagocytosis)
- About 25% of pregnant women are vaginal carriers for GBS
- GBS infections are acquired by neonates at the time of birth
- Diseases caused by S. agalactiae:

1- Neonatal sepsis which may manifest as pneumonia, septicemia, neonatal meningitis

2- Serious infections in adults e.g. pneumonia and endocarditis particular cancer and diabetic patients.

- Prevention:

1. Routine screening for S. agalactice in pregnant women at the end the 3 trimester (35 or 36 weeks).
2. Colonized mothers are given ampicillin during delivery (intrapartum) to reduce neonatal sepsis.

Alpha-Haemolytic Streptococci

A. Viridans Streptococci:

1. Normal inhabitants 2. oral cavity 3. gastrointestinal (GIT) 4. genital tract

2. virulent factor:- **glycocalyx**

pathogenesis:

1. Viridans streptococci play a significant role in **dental caries (S. mutans)**
2. 50% of all cases of **subacute bacterial endocarditis (SBE)**

- SBE may occur when dental manipulations or trauma to mucosa of upper respiratory tract, e.g. tonsillectomy or oral surgery

Laboratory diagnosis of SBE

- blood culture technique with subculture on blood agar
- 5-10 ml blood + 50-100 ml broth, subculture on blood agar at 37°C for 48 hours/ 10 days
- The isolated organism should be discriminated from S. pneumoniae

Treatment

- Viridans streptococci are relatively resistant to penicillin
- combination of penicillin and gentamicin

Prevention

A single large dose of **ampicillin or amoxicillin** should be given to patients with abnormal heart valves prior to dental procedures or oral surgery to prevent endocarditis

2. Streptococcus pneumoniae (Pneumococci):

Morphology

- Gram-positive, lancet-shaped, capsulated
- arrange in pairs or shot chains (diplococci)

Culture

- Facultative anaerobe
- On blood agar, colonies show alpha-haemolysis

Virulence factors

- polysaccharide capsule:

1. Anti-phagocytic
2. antigenic
3. It divides the organism into about 90 different serotypes

Pathogenesis

1. Alcoholics
2. post-splenectomy
3. immunosuppressed
4. infants
5. elderly

Diseases

- Pneumonia
- meningitis
- otitis media
- Sinusitis
- Conjunctivitis
- endocarditis

Laboratory diagnosis

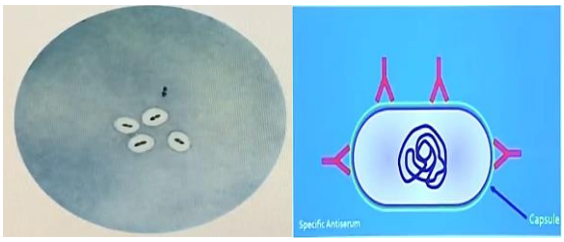
A-Specimens include sputum, CSF, ear or eye discharge (pus) and blood (endocarditis)

B-Direct detection

1- **Gram-stained smear**: Gram-positive, capsulated, diplococci. The capsule appears as an unstained zone around the organism.

2- **Quellung test**

- The capsule reacts with the specific antibody and can be seen under microscope to swell. This is the basis of the quellung test which is used to identify the pneumococcal serotypes



C-Cultivation

- Specimens other than the blood should be plated directly on blood agar incubated at 37°C
- Blood samples should be cultivated by the blood culture technique

D-Identification

- Colonies of viridans streptococci (non-pathogenic) are also encountered on blood agar while examining sputum for S. pneumoniae (pathogenic)
- Confusion occurs due to similarity in microscopic and colony morphology

Test	*S. pneumoniae*	*Viridans strept*
Growth inhibition by optochin	+	–
Solubility of colonies in bile (autolysin)	+	–
Capsular Ag detection	+	–
Quellung test	+	–

Treatment

Third generation cephalosporins (e.g. ceftriaxone) are the drugs of choice

Prevention

A. **Capsular polysaccharide vaccine:** It contains antigens from the most common 23 pneumococcal serotypes.

- The vaccine is used

1. after splenectomy
2. in elderly
3. immunosuppressed patients

- It is not effective in children less than 2 years of age who respond poorly to polysaccharide (thymus independent) antigen

B. **Protein conjugate vaccine:** It contains the capsular polysaccharide of the 13 most common pneumococccal serotypes conjugated to a protein carrier that makes the vaccine more effective in children less than 2 years of age

Harvesting questions

Q1) The following are virulence factors for S. pyogenes EXCEPT:

a) Fibronectin binding protein
b) M protein
c) Hyaluronic acid capsule
d) Coagulase

Q2) Acute rheumatic fever Should be followed by chemoprophylaxis to prevent further attacks

a) true
b) false

Q3) Which of the following procedures is most likely to reduce the inclean of group B streptococcal sepsis in infants:

a) Intrapartum antibiotic treatment
b) Use of a polysaccharide vaccine
c) Screening of pregnant females in the first trimester
d) Identification of possible high risk births
e) Antibiotic treatment of the newborn

Q4) After extraction of a tooth, a student with history of congenital heart disease was diagnosed as having subacute bacterial endocarditis. The most likely organism causing this infection is:

a) S. pyogenes
b) Streptococcus agalactiae
c) Streptococcus pneumonia
d) Viridans streptococci

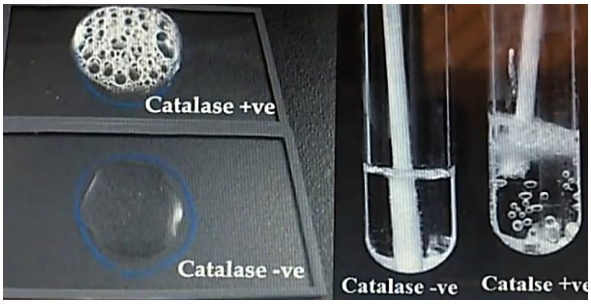
Q5) If a quellung test was done on the following bacterial isolates, which one would you expect to be positive?

a) S. pneumonia
b) S. pyogenes
c) Viridans streptococci
d) Streptococcus agalactiae

Lecture7

Staphylococcus

General characters of the genus Staphylococcus



1. Gram-positive spherical cocci, arranged in grape-like clusters
2. Opaque (colonies) on agar (**endo**pigment)
3. Catalase test (positive), different between :

- Staphylococci (+ve)
- Streptococci (-ve)

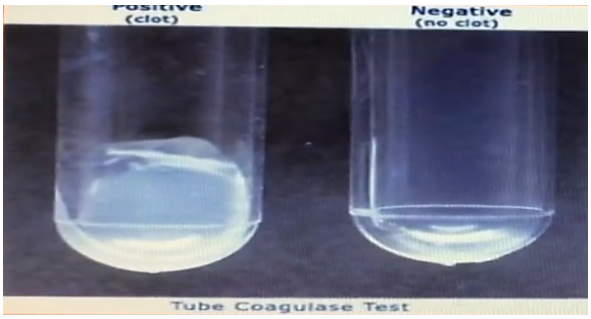
4. medically important species (at least 45 member), to different between **member** based on **coagulase test**

A. Coagulase-positive

- S. aureus (greatest pathogenic)

B. Coagulase-negative, which are far less pathogenic

- S. epidermidis
- S. Saprophyticus



Staphylococcus aureus (S. aureus)(aureus = golden yellow)

Morphology (as above)

1. Non moile
2. Non spore forming bacteria
3. Usually non capsulated, some strain capsulated

Cultural characters

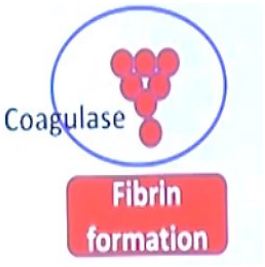
- S. aureus **is** a facultative anaerobe
- It is usually grown on

1. **Nutrient agar**: golden yellow colonies due to produces endopigment
2. **Blood agar** producing complete (β-) haemolysis due to production of haemolysins
3. **Mannitol salt agar** producing yellow colonies due to mannitol fermentation

- S. aureus (7.5-10 % salt tolerant), usually 0.5% salt in media
- This medium facilitates isolation of S. aureus (salt tolerant) from specimens contaminated by other bacteria

Virulence factors and pathogenesis

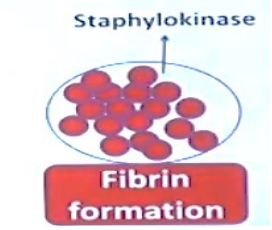
1. **The clumping factor** (fibrinogen-binding protein) (adhesion): This is an important adhesion that leads to attachment of the organism to traumatized tissue and blood clots.



2. **Staphylocoagulase:** (fibrin barrier)

- Coagulase has the ability to convert plasma fibrinogen to fibrin. By this mechanism:
    a) Protection from phagocytic and immune defences.
    b) Localization of infection

3. **Invasins:** (promote bacterial spread in tissues)

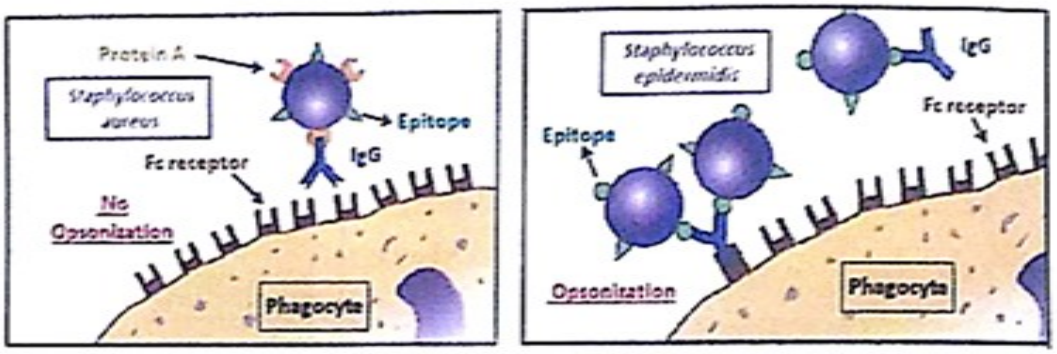


    A. Staphylokinase
    B. Leucocidin
    C. hyaluronidase

4. **Haemolysins:** These are pore-forming toxins that lyse host cell membranes They cause haemolysis on blood agar.

5. **Protein A** (antiphagocytic)

- It is present on surface of S. aureus.
- IgG (two portion FC bind to phagocytic cell, Fab binding to microb)
- It binds non-specifically to the FC portion of IgG leading to inhibition of opsonization.



Role of protein A

6. **Exotoxins**

a. Enterotoxins (food poisoning)
b. Epidermolytic (exfoliatin) toxins responsible for staphylococcal scalded skin syndrome (SSSS)
c. Toxic shock syndrome toxin-1 (TSST-1)

Staphylococcus aureus Diseases

- Staphylococci usually inhabit the skin (especially the perineum) and mucosa.
- 40-50 % human carriage in mucosal nose

A-Pyogenic diseases (pyo = pus)

I. **Localized skin infections** are the most commonly

a. Folliculitis, furuncles or abscesses

b. wound infections (post traumatic, post-operative)

II. **Staphylococcal pneumonia** is a frequent complication of

- viral infections (e.g. measles or influenza).
- Secondary bacteria infection

III. **Invasive conditions** (immuno-compromised individuals).

- Invasion of bloodstream (bacteraemia) and spread to numerous body sites lead to osteomyelitis, endocarditis, meningitis and septicaemia (fatal)

B-Toxin-mediated diseases

I. Staphylococcal food poisoning:

- It is the commonest type of bacterial food poisoning

1. protein-rich food like mayonnaise, milk and its products (e.g. ice cream)
2. carbohydrate-rich food e.g. pasta, cake

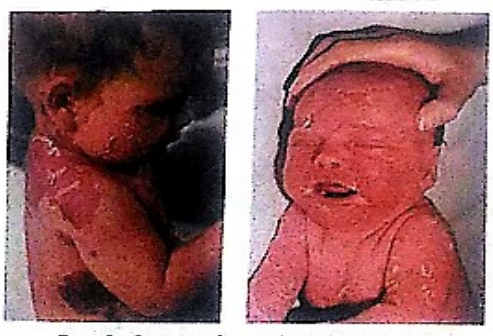
- There are 15 types of enterotoxins (A-E and G-P)
- produced by 50% of S. aureus strains.
- Staphylococcal enterotoxins do not change the characters of food (taste, colour or odour)
- Incubation period is short (1-6 hours)
- It manifests as violent vomiting and diarrhoea, usually **without fever**.
- It is usually **self-limited** (24 hours).

II. Toxic shock syndrome (TSS):

- TSS is due to infection or colonization by TSST1-producing S. aureus (20%) (F type).
- It was first described in young menstruating females who use vaginal tampons that are left in place for extended period.
- The syndrome can also occur in any individual (male, female, children)
- The disease is characterized by sudden onset of high fever, diarrhoea. vomiting and red rash. Hypotension with cardiac and renal failure may occur
- The mortality rate may reach 10-15%.

III. Staphylococcal scalded skin syndrome (SSSS):

- It occurs in neonates and children under 5 years of age.
- It follows infections caused by S. aureus that produces exfoliatin toxins.
- moist, red, scalded dermis.



Staphylococcal scalded skin syndrome

Laboratory diagnosis

A. Specimens may include pus, sputum, CSF, blood (bacteraemia, septicaemia and endocarditis) ....etc.

B. Direct detection in Gram-stained smears: Gram-positive cocci are seen in clusters in association with pus cells. Microscopy cannot discriminate staphylococcal species.

C. Cultivation

1. Specimens other than the blood should be plated directly onto
- **Nutrient agar**
- **Blood agar**
- **Mannitol salt agar**
2. Blood samples should be cultivated by the **blood culture technique**.

D. Identification

- After 24h incubation, the growth should be examined for colony morphology, Gram stain and catalase production. S. aureus is identified as follows:

1. **On blood agar**: golden yellow colonies surrounded by complete (β-) haemolysis due to production of haemolysins
2. **On mannitol salt agar:** producing yellow colonies due to mannitol fermentation.

➢ This medium facilitates isolation of S. aureus (salt tolerant) from specimens contaminated by other bacteria.

3. **Gram-stained film:** Gram positive cocci in clusters.
4. **Biochemical tests:**

a) **Catalase test:**

- Staphylococci (+ve)
- Streptococci (-ve)

b) **Coagulase test(free):** positive (result for hours)

c) **Clumping factor test(bound)** positive (results for second)

In case of food poisoning:

1. Specimens: tested for the causative S. aureus (selective media such as mannitol salt agar)
   - food remnants
   - vomitus and/or faeces
2. Detection of enterotoxin production by ELISA.

In case of toxic shock syndrome:

The diagnosis usually depends on:

1. Specimens: tested for the causative S. aureus (selective media such as mannitol salt agar)
   - tampons or faeces vagina swab
2. Detection of TSST-1 in the blood by ELISA.

Prevention and control (no vaccine)

- hygiene measure (especially hand washing)
- aseptic techniques

Treatment and Antibiotic Susceptibilities

- Abscesses (first surgical drainage, then antibiotic therapy)
- Systemic infections (antibiotic treatment)

Antibiotic-resistance patterns of S. aureus:

1. Penicillin-resistant S. aureus

- 95% of S. aureus strains are resistant to penicillin (penicillinase)
- semi-synthetic penicillins e.g. oxacillin and methicillin

2. Methicillin-resistant S. aureus (MRSA)

- There is a change in the penicillin-binding protein (PBP) which is the binding site for the antibiotic on the organism's cell wall. This type of resistance is due to the presence of mec-A gene on the chromosome of MRSA.
- MRSA isolates are often multiresistant to other antibiotics.
- Vancomycin is used as the drug of choice for treatment of MRSA infections.

3. Vancomycin-resistant S. aureus (VRSA)

- some strains of MRSA displayed intermediate (VISA) or full resistance (VRSA) to vancomycin
- The new antibiotics linezolid and streptogramins are used for treatment of infections not responding to vancomycin.

Coagulase Negative Staphylococci

I. S. epidermidis

Morphology and Cultural Characteristics

S. epidermidis is similar to S. aureus except in:

- **Nutrient agar**:- white colonies
- **Blood agar**:- white non-haemolytic colonies
- **Mannitol salt agar**:- mannitol non-fermenter.

Virulence factors

1. Glycocalyx (or slime):
   - extracellular polysaccharide
   - enables organism to colonize prosthetic devices
2. Biofilm: (aggregation of microorganism)

- adhere to surface
- Biofilms protect bacteria
- facilitate exchange of genetic material

Pathogenesis

- normal skin flora
- Almost all infections are endogenous
- device-related infections
  1. catheter (UTI)
  2. prosthetic valve endocarditis
  3. prosthetic joints
  4. surgical wound infections.

Laboratory diagnosis: It is sensitive to novobiocin

Treatment

- multi-resistant to antibiotics.
- The infections usually occur in prosthetic devices

II. S. saprophyticus

Morphology and cultural characteristics

S. saprophyticus is similar to S. epidermidis.

Pathogenesis

- the normal flora of human skin and mucosa of genitourinary tract.
- It may spread to urinary tract in colonized young femel sexually-active
- honeymoon cystitis
- endogenous infection

Laboratory diagnosis

Staph. saprophyticus is similar to S. epidermidis except in being novoblocin resistant.

Treatment

- Quinolones are the drugs of choice

MCQs:

1. The localized nature of S. aureus lesions is due to:

a. Adhesins
b. Protein A
c. Staphylocoagulase
d. Catalase

2. The following statements about S. aureus food poisoning are true EXCEPT:

a. It is caused by enterotoxins
b. The source of contamination is usually a carrier
c. The incubation period is 24-36 hours
d. Food contains preformed toxin

3. MRSA isolates are treated empirically by:

a. Erythromycin
b. Vancomycin
c. Clindamycin
d. Cephalosporins
e. Tetracycline

4. The most important factor enabling S. epidermidis to colonize prosthetic devices is:

a. Production of coagulase
b. Resistance to many antibiotics
c. Production of glycocalyx
d. Production of exotoxin
e. Production of clumping factor

Lecture9

Mycobacterium

Characters of the genus Mycobacterium (Acid-fast bacilli)

1. Acid-fastness:

- The cell wall of mycobacteria is rich in lipids (especially mycolic acid), stained with special techniques such as **Ziehl-Neelsen method** (20% H2SO4)
- Termed **acid-fast** due to resist decolourization even by acidic solutions

2. Slow rate of growth:

- to produce visible colonies on sold media

1. Slow growers require more than 7 days (**up to 8 weeks**)
2. rapid growers require less than 7 days (**6 days**)

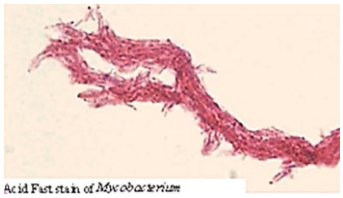
3. Obligate aerobe

Members of the genus Mycobacterium

1. **Mycobacterium tuberculosis complex (typical T.B):**
   - M. tuberculosis
   - M. bovis
   - M. africanum
   - M. microti
2. **Nontuberculous mycobacteria (NTM)**
3. **Mycobacterium leprae: (leprosy)**
4. **Saprophytic mycobacteria**

Mycobacterium tuberculosis

Morphology


Acid Fast stain of *Mycobacterium*

- Slender, thin, delicate
- acid-fast bacilli
- They appear pink when stained with the Ziehl-Neelsen
- **Serpentine cords:** is characteristic when smears prepared from cultures (especially fluid media), due to virulent strains (cord factor)

The high concentration of lipids in the cell wall of M. tuberculosis has been associated with:

1. Impermeability to stains and dyes
2. Resistance to drying
3. Resistance to many antibiotics
4. Resistance to killing by acidic and alkaline compounds
5. Resistance to lysis via complement deposition
6. Ability to survive inside macrophages

Cultural characters

M. tuberculosis requires **special media** for growth such as **Lowenstein-Jensen (L-J) medium**

- an egg-based medium
- malachite green dye (**toxic to respiratory flora**)

Cell wall structure

- **unique** to the Mycobacterium species, It has peptidoglycan layer which is similar to that of Gram-positive bacteria
- In addition, it comprises the following lipids:

1. **Mycolic acids:** are long chain fatty acids containing 60 to 90 carbons
2. **Cord factor:** inhibits leucocyte migration and disrupts mitochondrial respiration
3. **Mycobacterial sulfolipids:** inhibit phagolysosome formation

Susceptibility to physical and chemical agents

1. Tubercle bacilli are killed by:
   - Heating at 55°C for 1 h, autoclaving, pasteurization (M. bovis) and sunlight
   - Intermediate level disinfectants such as **ethyl and isopropyl alcohols and chlorine**
2. They survive for many weeks when dried (evaporation) in sputum smeared on clothing and in dust
3. They are resistant to acids and alkalis, , an **important feature, which is used in cultivation procedures from contaminated specimens**

Human Tuberculosis

- The number of cases with TB has increased after the start of **AIDS pandemic**
- Bacteria that cause the new outbreaks may be **multidrug-resistant**

Mode of transmission

➢ Aerosol (airborne) (droplet nuclei) **or** sputum (dust particle), **bacilli can survive for several weeks**

- **Droplet:** size **more than 5 micrometer**
- **Droplet nuclei:** size **less than 5 micrometer**, the commonest mode of infection due to inhalation of droplet nuclei carried by air from a patient with open pulmonary tuberculosis

➢ Ingestion of milk of infected animals (contaminated with M. bovis) (intestinal infection)

Pathogenesis

A. **Local infection**
   - The droplet nuclei (bacilli), that engulfed by alveolar macrophages
   - The remaining bacilli are able to survive
   - Accumulation bacilli kill macrophage (virulent factor sulfolipids)
   - Two week later → regional lymph → primary lesion → Gohn's complex (exudate lung lesion with drainage lymphnode)

B. **Transient bacteremia:** Bacillemia phase (few days later)

C. **granuloma formation (granuloma tubercle)**
   - 4-6 weeks after infection (diffusion to tissue such as brain, bone, kidney, lung) (**obligate aerobes**)
   - Within the granuloma, M. tuberculosis can survive in small numbers in a relatively dormant state (**latent** tuberculosis **infection**).
   - No case (**without manifestation**), no carrier (**noninfectious**)

D. Outcome of primary infection

- Primary tuberculosis may follow one of 2 courses:

1. **Latent tuberculosis infection (diagnosis, skin test after 4-6 weeks post infection)**
   - without development of symptoms
   - This occurs in about 90% of the infected people
2. **Active tuberculosis disease: (manifestation)**

- In the remaining 10% of people
- TB bacilli overcome the immune system and begin to multiply
- Symptoms of disease include general malaise, fatigue, night sweats and fever, along with persistent cough, and maybe bloody sputum
- Tuberculosis may affect other systems (any organ) , e.g. tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis

Characters	Latent TB infection	Pulmonary TB disease
M. tuberculosis present	Yes	Yes
Tuberculin skin or Quantiferon test	Positive (cell mediated immunity)	Positive (cell mediated immunity)
Sputum smears and cultures	Negative (**dormant, close lesion**)	Positive (**open lesion**)
Symptoms	No symptoms (**no case**)	Cough, fever, weight loss
Infectivity	Not infectious (**no carrier**)	Often infectious

E. Reactivation

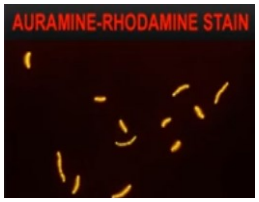
- immunosuppression (AIDS)
- Reactivation usually occurs within 2 years after initial infection in about 5% of individuals with latent TB infection
- Bacteria that cause the new outbreaks may be multidrug-resistant

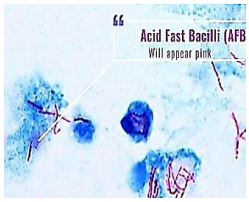
Laboratory diagnosis

1. Laboratory diagnosis of open (active) pulmonary tuberculosis

A. Specimens (sputum)

B. Direct detection





a) Smears

- Ziehl-Neelsen (Z-N) method (confirmed)
- Fluorochrome staining with the auramine-rhodamine stain (screening)

b) Molecular methods: PCR

C. Processing of sputum (viscous) by

1. liquefaction(N-acetyl-L-cysteine)
2. decontamination (NaOH)
3. concentration (centrifuge)

D. Cultivation (special media)

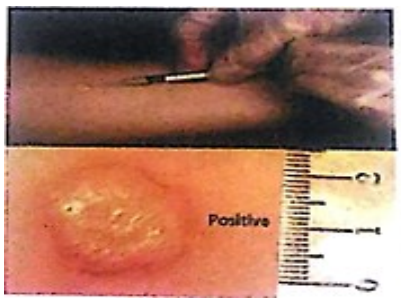
- Conventional culture media such as Lowenstein-Jensen (L-J) medium (colonies appear after about 2-8 weeks of incubation)
- Fluid medium systems (detection of growth in 4 to 14 days)

E. Tuberculin Skin Test (TST) and Quantiferon tests

2. Laboratory diagnosis of extrapulmonary tuberculosis (CSF, urine,…)

- such as tuberculous meningitis, lymphadenitis and intestinal tuberculosis, specimens collected from sterile sites, e.g. CSF, urine do not require decontamination

3. Laboratory diagnosis of latent tuberculosis (Tuberculin Skin Test (TST) and Quantiferon tests)



Treatment regimens of tuberculosis

1. Combination therapy: (Combination of 4 drugs)
   - prevent the emergence of drug resistant mutants
   - reduce the drug toxicity
2. Prolonged therapy
   - at least 6 months (8): (4 drugs X 2 weeks, then 2 drugs X 4 weeks)
   - this is because of:
     - the intracellular location of the organism
     - blocking of drug penetration by caseous material
     - the slow rate of growth of the organism
     - the metabolically inactive bacilli within the lesion
   - non-infectious within 2-3 weeks

Anti-tuberculous drugs include:

- First-line drugs
  1. isoniazid
  2. rifampin
  3. pyrazinamide
  4. ethambutol
  5. streptomycin
- Second-line drugs (only used if resistance first-line drugs): fluoroquinolones, para-aminosalicylic acid, ethionamide, cycloserine, capreomycin, kanamycin……. etc

Resistance patterns:

- Multidrug-resistance (MDR) means resistance of M. tuberculosis to both isoniazid and rifampin.
- Extensive (extreme) drug resistance (XDR) means MDR plus resistance to a fluoroquinolone and at least one additional drug

prevention and Control

A. General measures

- Early case finding and effective treatment
- Applying proper infection control measures in hospitals (e.g. use of N95 masks)
- Avoid overcrowding
- Better housing and housing and nutrition to improve host resistance
- Pasteurization or boiling of milk

B. Treatment of latent infection: To reduce the risk of progression to active tuberculosis (isoniazid is given for 6-9 months)

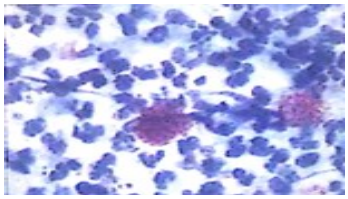
- Recent converters
- Tuberculin or Quantiferon positive (immunosuppression)

C. Bacille Calmette-Guérin vaccine (BCG vaccine):

- It is a live attenuated vaccine prepared from M. bovis
- protection against tuberculous meningitis and disseminated tuberculosis

Mycobacterium leprae (Hansen's bacilli)

Morphology



- Bundles, groups, (globi)
- Modified Ziehl-Neelsen method (5% H2SO4), decolourizing agent is modified, is necessary to avoid overdecolourizing

Culture

- not cultured in vitro
- It is considered an obligate intracellular pathogen

Leprosy (Hansen's disease)

- is a chronic infectious disease

Pathogenesis and clinical manifestations

- **mode of transmission**: is not certain

1. infection requires prolonged and close contact skin lesions with patients
2. Infection may be aerosol inhalation

- **incubation period**: years; this is due to:

➢ low infectivity
➢ long generation time (slow rate of growth), (13 hours)

- Leprosy bacilli grow best at low temperature (optimally 30°C); therefore, the skin and superficial nerves are preferentially affected
- The disease presents in two basic forms:
  1. tuberculoid leprosy (TL)
  2. lepromatous leprosy (LL)

Laboratory diagnosis

- Clinically
- laboratory diagnosis (confirmation)

A. Specimen: Slit skin smears, skin biopsy, or scrapings from the nasal mucosa
B. Direct detection: Smears stained with modified Z-N method, is enough diagnosis

Treatment

- Prolonged
- Multidrug therapy

1.LL: Triple therapy (2 years)
- Rifampicin, clofazimine and dapsone

2.TL: a combination therapy (6 months)
- dapsone and rifampicin
- Nerve damage (irreversible)

MCQs

1) Mycobacteria are acid-fast positive bacteria because of:

a) the presence of lipopolysaccharide in the bacterial cell wall
b) the presence of mycolic acid in the bacterial cell wall
c) the presence of lipids
d) Both B and C options above

2) Which of the following first-line antibiotics are usually resistant to *Mycobacterium tuberculosis*?

a) Isoniazid and ciprofloxacin

b) Isoniazid and Rifampin

c) Rifampin and ciprofloxacin

d) Rifampin and streptomycin

3) All of the following are the symptoms of pulmonary tuberculosis, EXCEPT?

a) Weakness and fatigue

b) Decreased body temperature

c) Weight loss

d) Severe prolonged cough with sputum or blood

4) All of the given are the distinguishing characteristics of *Mycobacterium leprae*, EXCEPT?

a) It is an acid-fast bacillus

b) It cannot be isolated in-vitro culture method

c) It is a human and as well as animal pathogen

d) It can be isolated by only in-vivo culture method

5) Which one of the following acid-fast rod bacilli can take up to ten years for its growth in host cells and causes skin infections?

a) *Mycobacterium tuberculosis*

b) *Mycobacterium avium* complex

c) *Mycobacterium leprae*

d) *Mycobacterium bovis*

6) Other than *Mycobacterium tuberculosis* which of the following bacteria causes tuberculosis infection in animals and can be transmitted to humans by consumption of milk and other animal products?

a) *Mycobacterium tuberculosis*

b) *Mycobacterium leprae*

c) *Mycobacterium bovis*

d) *None of the above*

Lecture8

Spore-forming Gram positive Bacilli

1. Aerobic or facultative anaerobic: **Bacillus**
2. Anaerobic: **Clostridium**

Bacillus

The most important species

1. **Bacillus Anthracis**: cause anthrax
2. **Bacillus Cereus**: cause food poisoning

A. Bacillus Anthracis (Anthrax)

Morphology

- Gram Positive, spore forming
- large, rectangular bacilli, arranged in chain
- spore (SSSS)

1. Size (Small)
2. Shape (oval)
3. site (Central)
4. stain (don't stain with gram stain)

❖ Ziehl-Neelsen Stain 0.5% H_2SO_4 (decolorizing)

❖ WHO (up to 40 years)

- Capsule Polypeptide

✓ **McFadyean reaction**: polypeptide capsule is detected in smears from infected tissues

✓ **polychrome methylene blue**: organisms (blue rods), capsular (pink-stained)

Cultures

1. Simple media
2. Enriched media (non-hemolysis)

pathogenesis

two virulence factors:

A. Capsule

B. anthrax Toxins:

1. Oedema factor (OF) (PA + OF ⟶ Odema toxin)
2. lethal factor (LF) (PA + LF ⟶ Lethal toxin)
3. protective Antigen (PA) (B Subunit)

- Odema toxin (Accumulation of fluid extracellular)
- Lethal toxin (necrosis, death cell)

Clinical manifestations: three forms (route of infection)

- zoonotic
- No person to person transmission

1. Cutaneous anthrax (malignant Pustule)

- Spores from the soil or infected animal enter skin abrasion, usually on an exposed area
- Typical lesion : ulcer with black eschar
- Antibiotics
- Untreated cases (blood stream, septicemia)

2. Pulmonary anthrax: (Inhalation)
3. Intestine anthrax (rarely)

Diagnosis

1. Clinical
2. McFadyean reaction

Treatment: Ciprofloxacin

Prevention: vaccine (purified protective antigen (PA))

B. Bacillus Cereus: Cereal

Comparison between types of B. cereus food poisoning

	The emetic form	The diarrheal form
Incubation Period	short (1-6 h)	Long (8-48h)
Manifestations	Vomiting, abdominal cramps	Diarrhea, abdominal cramps
Etiology	Performed ST enterotoxin (heat stable) (emetic toxin)	Performed LT enterotoxin (heat labile) (diarrhar toxin)
Action of toxin	Irritation of gastric mucosa	Activation of intestinal adenyl cyclase
Associated food	rice, Chinese restaurants	Meat dishes
Similarity of food poisoning causes by	S. aureus	C. perfringens

Clostridium

Characters of genus

1. large, G(+), bacilli
2. spore forming
3. Anaerobic
4. Most species are motile

Natural habitat

1. Soil
2. intestinal tract of human and animal

The most important species:

1. C. perfringens (gas gangrene, food poisoning)
2. C. Tetani (tetanus)
3. C. botulinum (botulism)
4. C. difficile (pseudo member colitis)

Water pollution

1. E. Fecalis
2. C. perfringens
3. E. Coli

A. Clostridium perfringens (C. perfringens)

Clostridia Causes Gas Gangrene

Sacchrolytic Clostridia	Proteolytic Clostridia
Cl. Perfringens (80%)	C. histolyticum
C. novyi	C. fallax
C. Septicum	C. bifermentans

Morphology

- large. Gram (+), bacilli, spore forming

Culture

1. anaerobic
2. on blood agar: unique double Zone of hemolysis (α, β Toxins)
3. On egg Yolk agar:

- Lecithinese (α-toxin)
- Nagler's reaction (opaque Zone)

Virulence factors

- At least 12 exotoxin, the most important α toxin
- These exotoxin:

1. haemolysis
2. cytotoxic
3. necrotic and cell death

Gas Gangrene (Clostridium perfringens 80%)

1. Clostridia myonecrosis
2. A cute disease with very poor prognosis and fetal out Come
3. All Clostridia wounds infection occur in anaerobic tissues (impairs blood supply):

- Trauma
- foreign body
- malignancy
- Surgery

4. organism and it's spore found in soil and humans animals feces
5. spore gain access to traumatized tissues by Contamination these sources
6. lack of oxygenation allow germination of spore and growth of Clostridia
7. The organism multiply in subcutaneous tissues cause anaerobic cellulitis (local)
8. The organism invade deeper tissue muscle produce exotoxin (α toxin), and cause massive cell death
9. other enzyme (e.g.. hyaluronidase and collagenase) which facilitate the spread of infection
10. fermentation of tissue carbohydrate cause rapidly accumulation gases cause crepitation (clinically)
11. the muscle becomes frankly gangrenous, black and extremely friable
12. If the toxins enter the bloodstream (massive haemolysis, renal failure and eventually death)

Diagnosis

- Clinically

1. Specimen: depth of wound
2. Gram Stain smear (pus(dead leukocyte)) , clostridia toxin lysis pus (appear like fluid exudate)
3. Cultivation (only anaerobic)
4. Identification

Treatment and prevention

1. Surgical debridement to remove foreign materials
2. Antibiotic: Penicillin, Ampicillin
3. Hyperbaric oxygen chamber (respiratory failed)
4. Anti-Alpha Tokin (not effect)

C. perfringens food poisoning

1. Ingestion of large number of organism in Contaminated food (meat or meat product)
2. Enterotoxin (LT)
3. Incubation Period (8-48h)
4. Diarrhea, abdominal crimps
5. Self-limiting
6. Not treatment is require

B. Clostridium Tetani (Tetanus)

- Clostridium Tetani: Tetanus
- found in intestinal tract and faces of various animals
- Human Intestine (0-25%)
- Spore of C. tetani abundant in soil (heavily manures soil)

Morphology

- large, G (+), bacilli
- spore: (drum stick)

1. Size (large, bulging)
2. Shape (spherical)
3. Site (terminal)
4. Stain (ziehl neelsen stain)

Culture (anerobic)

- on simple media (cooked meat media)
- on blood agar (B-hemolysis)

Virulence factor

- exotoxin (tetanospasmin) (neurotoxin)

Tetanus

Pathogenesis and Clinical manifestation

- A/B modal (A and B subunits)
- Incubation period 4 days - several weeks
  - ✓ severity of the wound
  - ✓ proximity to the brain
- Lack Jaw (Trismus)
- Arching of back
- Ex-aggressive reflexing → (nose - Light)
  - Death (chest Paralysis)
  1. 40% adult
  2. 90% neonatal
- Neonatal Tetanus (umbilical stump)

Diagnosis

- Clinically (antitoxin)
- Laboratory diagnosis (confirmation): specimen (depth of wound)

Treatment

1. Human tetanus immunoglobulin (HTIG): (antitoxin)
2. local debridement of wound
3. Metronidazole or penicillin
4. Supportive measures

prevention and Control

- Prophylactic immunization is only way to control Tetanus
- single antigenic Type (immunization effective)
- Tetanus Toxoid (aluminum salt adjuvant)
- The Toxoid is given to:

1. infants (2,4,6 month, 18 month, school age)

   ✓ The triple vaccine (DPT) (diphtheria, pertussis, tetanus)
   ✓ Booster doses (Td): in adult

2. Wound individual
3. Pregnant female (two dose)
4. People high risk group (military personnel)

Antitoxin

1. No history of vaccination
2. Last booster dost more than 10 years
3. If the wound grossly contaminant

C. Clostridium botulinum (botulism)

- C. botulinum is widely distributed in Soil and sediments of lake , ponds and decaying Vegetation.
- The intestinal tract of mammals, fish and birds

Morphology and Culture

1. Gram (+), large bacilli
2. spore forming
3. Anaerobic
4. On simple media (cooked meat media)
5. On blood agar

Virulence factors

- Botulinum toxins (neurotoxins (CNS))
- Several antigenic Types of C. botulinum Toxins (A, B and E)

- Type A toxin is the most potent toxin in existence (1 gm would be enough to kill 14,000 people if ingested or 1.25 million people if inhaled)

- A/B model

Pathogenesis

- spores, wide spread in soil, contaminated vegetables and meat (Canned food)
- Toxin is produced within canned food and ingested Performed (green bean, smoked salt Fish, mushroom, canned Salmon)
- The Toxin is absorbed in intestine and transport via blood Stream to reach peripheral neuromuscular Junction
- Toxin binds to neuron and prevent release acetylcholine, Produce (flaccid paralysis)

N.B.: Botox

1. wrinkles
2. spasmodic muscle (torticollis)

Clinical manifestations

A. Classic Botulism

- incubation Period (12-36h)
- The Cranial nerved are affected (blurred of vision, inability to swallow, and difficulty of speech's, followed by systemic paralysis of motor nerves)
- Mortality rate 15%

B. Infant Botulism

- ingestion of food supplements contain raw honey contaminated by C. botulinum
- infants (2 weeks and 6 months) before establishment of competing intestinal flora
- Sucking power weak, constipation, generalized weakness floppy baby syndrome

C. Wound botulism

Wound become contaminated with organism absorbed from the site drug addicts (Skin popping with heroin)

Diagnosis

- Clinically (antitoxin)
- Lab. for confirmation

1. Toxin (ELISA)
2. Gene (PCR)

Treatment

- Trivalent (A, B and E) antitoxin (with in 12h after ingestion)
- No reason to give antibiotics, except (wound and infant botulism)

Prevention

1. proper Sterilization of canned food
2. Boiling (10 min. or cooking) (L.T.)
3. swollen canned must be discarded

D. Clostridium. difficile

- C. difficile is most common cause (antibiotic associated diarrhea)
- C. difficile is a minor component of the normal flora of the large intestine 3%, hospitalized people 30%
- The hands of hospital personals play an important role in faeco-oral transmission

Morphology and Culture

- large bacilli, G (+), spore forming, anaerobic

Virulence factors and pathogenesis

- Toxin A (enterotoxin) (diarrhea)
- Toxin B (cytotoxic, that kill colonic mucosal cells)
- Severity case Pseudomembrane colitis

Diagnosis

- Clinical
- Toxin (ELISA)
- Genes (PCR)

Treatment and prevention

1. with drawls of the causative antibiotic
2. treatment with antic difficile drugs
   - oral vancomycin or metronadzole
3. Correction of dehydration and electronic imbalance
4. Anti-diarrheal agent Not be taken
5. Restoration of the patient's colonic flora by faecal enema from a normal individual

MCQs:

Q1) The following statements concerning B. anthracis are correct EXCEPT:

A. It is a Gram-positive spore forming bacillus.
B. It causes a zoonotic disease.
C. It is a typical biological weapon.
D. The toxin is its only virulence factor.
E. It can cause pneumonia or skin lesions.

Q2) The emetic form of B. cereus food poisoning is characterized by the following EXCEPT:

A. It resembles S. aureus food poisoning.
B. It has a short incubation period.
C. The incriminated food is usually fried rice.
D. It is due to heat labile enterotoxin.
E. It is manifested by vomiting and abdominal cramps.

Q3) The following is true about Clostridium perfringens **EXCEPT**:

A. It causes gas gangrene.
B. It causes food poisoning.
C. It produces an exotoxin that degrades lecithin.
D. Endotoxin is a virulence factor of the organism.
E. It is one of the indicators of faecal pollution of water.

Q4) Active immunization against tetanus is given to the following **EXCEPT**:

A. Pregnant females
B. Infants in the first year of life
C. Military personnel
D. Wounded individuals with history of vaccination within 2 years
E. Routinely every 10 years

Q5) Symptoms of botulism are due to:

A. Invasion of the gut epithelium by C. botulinum
B. Secretion of an enterotoxin
C. Ingestion of a neurotoxin
D. Endotoxic shock
E. Activation of cyclic AMP

Q6) A patient presents with severe colitis associated with an overgrowth of C. difficile in the bowel. The most likely cause of this condition is:

A. Botulinum food poisoning
B. A stomach ulcer
C. Compromised immune system
D. Antibiotic therapy
E. Mechanical blockage of the large intestine