





Micro L 1 :Introduction to medical microbiology

Microbiology	Microbiology is the science that deals with microorganisms (mikros = small \ bios = life \ Logos = science)
Microorganisms	Microorganisms are small living organisms that cannot be seen by naked eye except by microscope

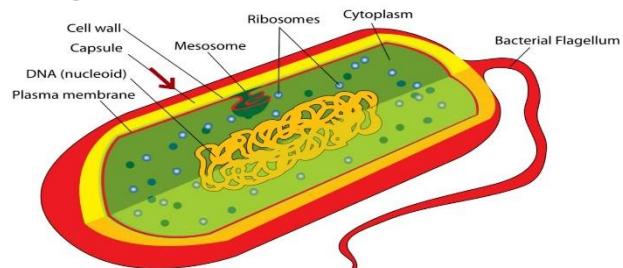
Eukaryotes and prokaryotes

There are two major divisions of cellular organisms	
Prokaryotes	Eukaryotes
(Pro = premature or primitive, Karyon = nucleus)	(eu = true, karyon = nucleus)
Cells with premature nucleus (single naked chromosome without nuclear membrane) e.g. bacteria and rickettsia	Cells with true nucleus which contain a nuclear membrane, nucleoli, and multiple chromosomes within the nucleus e.g. Fungi

STRUCTURE OF THE BACTERIAL CELL

Any bacterial cell is composed of the following structures (Essential structures):

1. Cell wall.
2. Cytoplasmic membrane
3. Cytoplasm.
4. Nuclear body.



Some (Not all) bacteria may contain one or more of the following structures:

1. Capsule.
2. Flagella
3. Fimbria (pili).
4. Inclusion granule



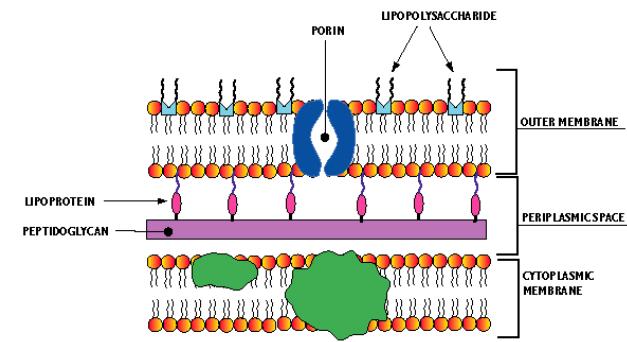
CELL WALL

Definition: The outer covering layer of the bacterial cell (outside the cytoplasmic membrane). It is relatively rigid with some elasticity.

- It is composed of :

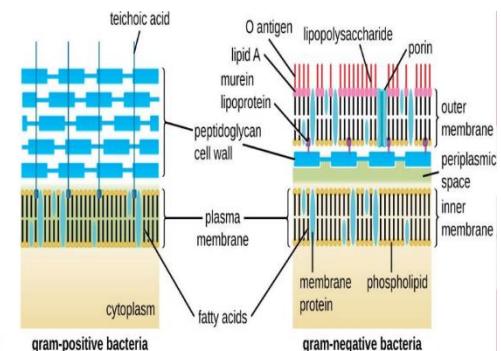
(1) Basic layer = peptidoglycan = mucopeptide.

- Formed of carbohydrate and protein.
 - a) Carbohydrate backbone is formed of N – acetyl glucosamine and N – acetyl muramic acid.
 - b) The protein part is formed of tetrapeptide side chains connected to N acetyl muramic acid
- It is responsible for the rigidity of cell wall.
- Forms 80% of Cell wall of gram positive bacteria and 20% of cell wall of gram negative.



2- Additional layer:

- Its structure varies according to the type of bacteria:
 - In Gram +ve bacteria it is formed of teichoic acid.
 - In Gram -ve bacteria it is formed of lipopolysaccharides
 - ✓ inner: highly toxic, lipid A
 - ✓ middle: polysaccharide core
 - ✓ outer: polysaccharide , somatic O antigen) and lipoproteins



Periplasmic space:

The space between the inner and outer membranes containing the peptidoglycan and is filled with gel containing enzymes such as β -lactamase that degrade β lactam drugs.



Function of the cell wall:

1. It maintains the shape of the bacteria.
2. Protection of bacterial cell against high osmotic pressure and outer surroundings.
3. It is antigenic (help in identification and stimulate the production of protective antibodies).
 - o Teichoic acid in gram +ve bacteria is major surface Ag.
 - o Lipopolysaccharide (LPS) in gram -ve bacteria is also major surface Ag (OAg).
4. Site of action of penicillin, cephalosporins and lysozymes.
5. Lipopolysaccharide layer in Gram – ve bacteria is an endotoxin.
6. Responsible for staining properties of bacteria

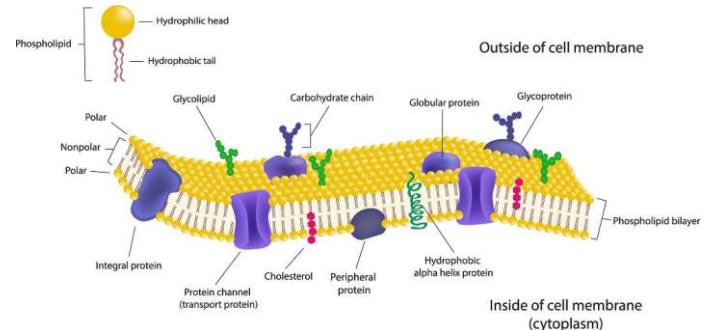
Abnormalities of the cell wall:

1. Protoplasts
2. Spheroplasts
3. L-forms
4. Mycoplasma

Quiz: List 4 functions of the cell wall

CYTOPLASMIC MEMBRANE

- o **Def:** Semipermeable, double layered membrane, just internal to the cell wall, and surrounding the cytoplasm.
- o **Composition:** Lipoprotein (70% protein and 30% phospholipids).
- o **Functions:**
 1. Selective permeability = selectively transport nutrients to the cell, and waste products outside the cell
 2. Active transport of ions and molecules to the inside of cells.
 3. It contains respiratory enzymes and pigments → oxidative phosphorylation → energy to the cell.
 4. Play a role in DNA replication.
 5. Play a role in cell wall synthesis.
 6. Excretion of pathogenicity proteins e.g. IgA protease.

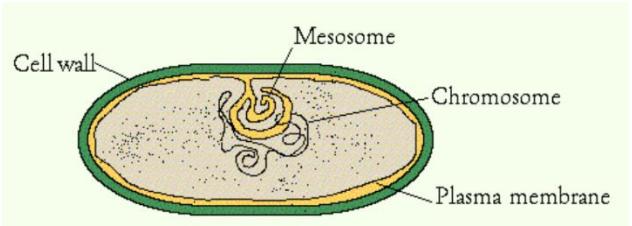




7. Excretion of hydrolytic exoenzymes which degrade the different nutrients into subunits small enough to penetrate the cytoplasmic membrane

MESOSOMES:

- These are invaginations of cytoplasmic membrane inside the cytoplasm of the cell.
- Seen in many (but not all) bacteria.
- More common in **Gram +ve bacteria**.
- May be central (septal) or marginal (lateral).



Functions:

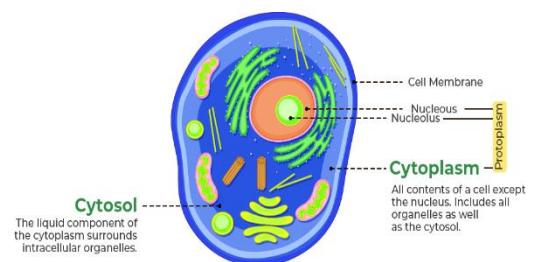
1. Increase surface area of cytoplasmic membrane.
2. May be site of respiratory enzymes.
3. Give attachment to the chromosome, so involved in cell division.
4. Excretion of enzymes as penicillinases.

QUIZ:

1. Differentiate between Gram positive and Gram negative bacterial cell wall.
2. Enumerate 4 functions of the cytoplasmic membrane.

THE CYTOPLASM

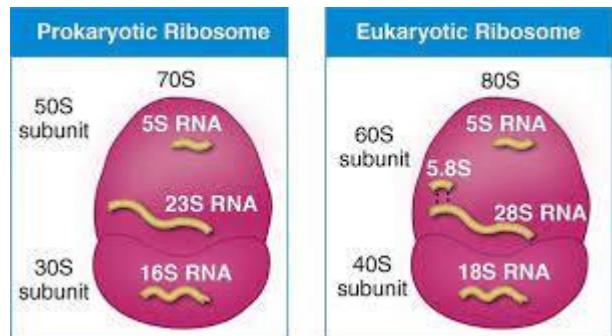
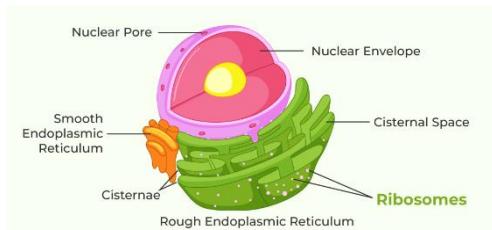
- It is a homogeneous soft gel mass inside the cell that contains:
 - ✓ Nuclear body
 - ✓ Ribosomes.
 - ✓ Storage granules
 - ✓ Enzymes.
 - ✓ Extrachromosomal DNA pieces e.g. plasmids.
- The cytoplasm of prokaryotes has **no mitochondria and no endoplasmic reticulum**





RIBOSOMES:

- They are complex minute structures inside the cytoplasm.
- Composed of RNA (60%) and proteins (40%).
- Bacterial ribosomes (70 S) are composed of 2 subunits a large one (50 S subunit) and a small one (30 S subunit).
- A group of ribosomes are called polysomes.
- They are **the site of translation of mRNA** during the process of protein synthesis.
- They differ from ribosomes of eukaryotic cells (80 S) and this allows some drugs (e.g. streptomycin) to affect bacterial cell but not human cell (patient).



THE NUCLEAR BODY

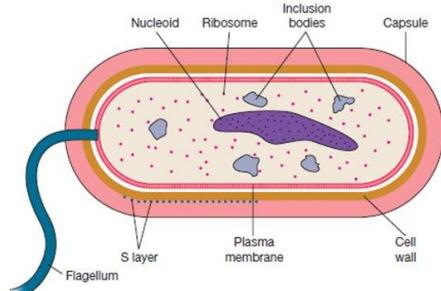
- It is a mass of DNA molecule coiled to form single chromosome (double stranded DNA molecule).
- There is no nuclear membrane and no nucleolus.
- It carries all the genetic information of the cell.
- It duplicates before cell division.

➤ Plasmid

- extrachromosomal double stranded circular DNA, replicating independent of the bacterial chromosome.

THE CAPSULE

- It is a gelatinous layer formed by some bacteria, and lies outside the cell wall.
- **Composition:** Polysaccharides in majority of bacteria e.g. pneumococcus and polypeptide in anthrax bacillus.
- **Demonstration:**
 - ✓ Capsule stain (Muri's stain).
 - ✓ Negative stain as India ink.
 - ✓ Unstained halo around the organism in Gram stain.
 - ✓ Electron microscopy.
 - ✓ Serology: e.g. Quellung reaction for pneumococcus.



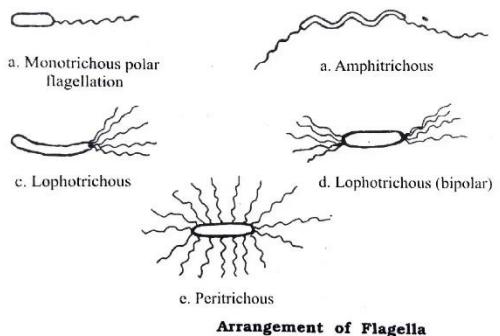
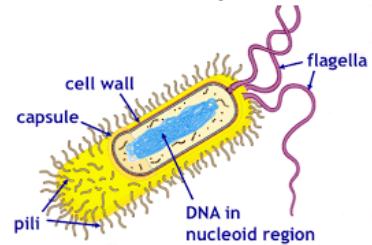


- **Function:**

1. Virulence factor = antiphagocytic factor protect pathogenic bacteria against phagocytosis.
2. Protect bacterial cells against bacteriophage, complement, lysozymes etc.
3. Antigenic (K Ag): help in identification and typing of bacteria e.g. pneumococci.
4. Vaccine preparation.
5. May help pathogenic bacteria in adherence to human tissues.

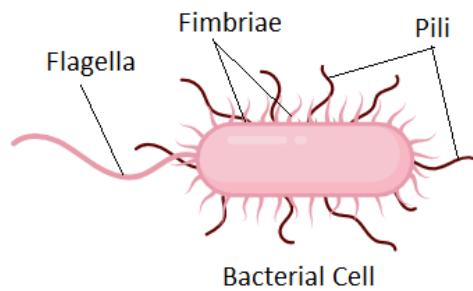
FLAGELLA

- They are long, hollow, filamentous appendages used as organ of motility.
- **Structure:** Formed of protein called flagellin.
- Arise from basal bodies in the cytoplasm.
- **Function:**
 1. Responsible for motility → pathogenesis.
 2. Antigenic (H antigen) → Identification of bacteria.



FIMBRIAE = PILI

- They are short, hair – like filaments found mainly on surface of Gram – ve organisms.
- **Structure:**
 - Formed of protein called (pilin).
 - Arise from the cytoplasmic membrane.
- **Function:**
 - ✓ Organ of adhesion.
 - ✓ Transfer of genetic material (conjugation).
 - ✓ Antigenic.





SPORES

- spores are highly resistant resting forms of some bacteria; formed on exposure to unfavorable conditions e.g. dryness, heat and depletion of nutrients.
- **Sporulation (sporogenesis)**: the process of formation of spores from vegetative cells.
- Sporulation is triggered by exposure to unfavorable conditions.
- **Germination**: opposite to sporulation i.e. formation of vegetative cells from spores in favorable conditions.
- **Medical importance:**
 - a) Resistance to heat and chemicals (bad environmental conditions).
 - b) Help identification of bacteria. e.g. spore of cl. tetani is terminal, rounded, bulging (drum stick appearance).

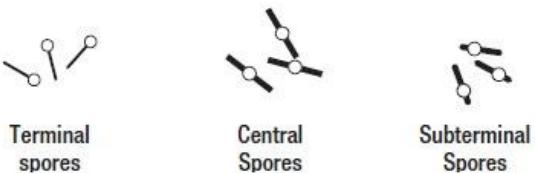
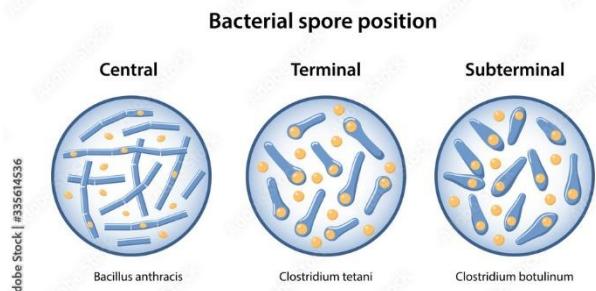
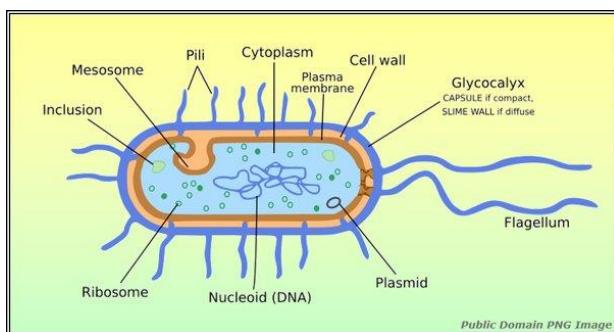


Figure 3.9: Position of spore in a vegetative cell.

GLYCOCALYX (SLIME LAYER)

- **Definition:** It is a thin coat ,that covers the surface of bacteria like a film.
- **Composition:** Polysaccharide.
- **Function:** It facilitates bacterial adhesion and colonization to various structures e.g. skin , heart valves , catheters and teeth (e.g. Strept. mutans that form plaque , the precursor of dental caries).





Quiz

1. What is the primary component responsible for the rigidity of the bacterial cell wall?
 - a) Teichoic acid
 - b) Lipopolysaccharides
 - c) Peptidoglycan
 - d) Flagellin
2. How does the membrane contribute to the transport of ions and molecules into the cell?
 - a) Passive diffusion
 - b) Osmosis
 - c) Active transport
 - d) Facilitated diffusion
3. What is the opposite process of sporulation, involving the formation of vegetative cells from spores?
 - a) Differentiation
 - b) Germination
 - c) Replication
 - d) Transformation



كل صعب
يعدى خلیان
فخور بكل
حاجه عدیتها
انت اکبر داعم
لنفسك.



Micro L2: Bacterial Physiology and Metabolism

Bacterial metabolism:

- Catabolism
- Anabolism
- The oxidation process
- Fermentation

Anabolism: is the building of complex molecules from numerous simple ones. The process utilize energy.

Catabolism: is the breakdown of complex molecules into numerous simple ones. This process produce energy

- Many bacteria secrete **enzymes** e.g. lipases, nucleases, proteinases and other hydrolytic enzymes.
- These breakdown **extracellular nutritive** material into simple **molecules** that are actively transported across the cytoplasmic membrane into bacterial cell.
- These are oxidized by bacteria to yield energy; and the degradation products are used to build up structural components and essential macromolecules for cell metabolism (**anabolism**).

The oxidation process:

- It involves a series of reactions in which electrons (**hydrogen**) are released in reaction and transferred to an electron acceptor.
- **The electron acceptor** is molecular oxygen in aerobic respiration or an inorganic compound, (e.g. nitrate) in anaerobic respiration
- The whole process is catalyzed by a set of enzymes and coenzymes similar to cytochrome system
- The energy that results from these reactions is stored as **high energy bonds** e.g. ATP to be used in anabolic process.

Fermentation:

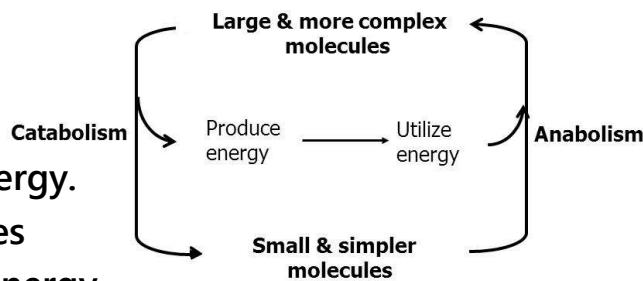
- Fermentation is a type of metabolism in which the substrate is metabolized **without the involvement of an exogenous oxidizing agent**.
- This is the process by which facultative bacteria generate ATP in absence of oxygen.
- It refers to breakdown of sugar to pyruvic then lactic acid. It is the mechanism by which facultative bacteria generate ATP in absence of oxygen.

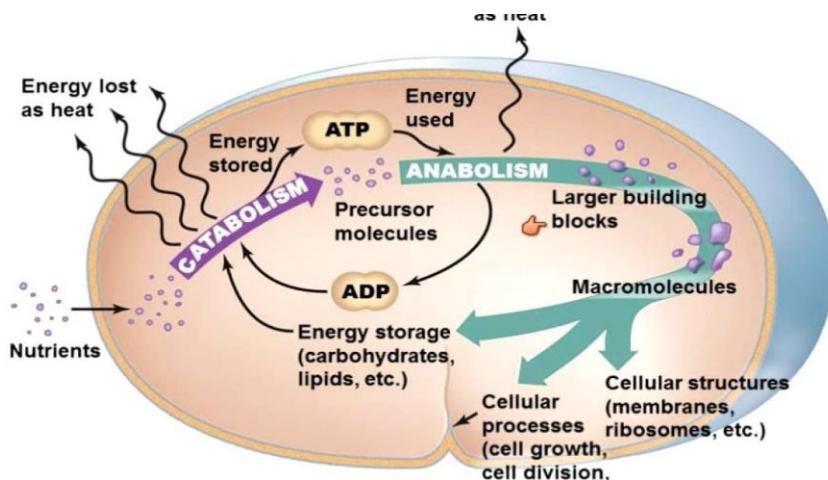


Metabolism

- Definition: metabolism – total of all chemical reactions occurring in a cell

Bacterial metabolism



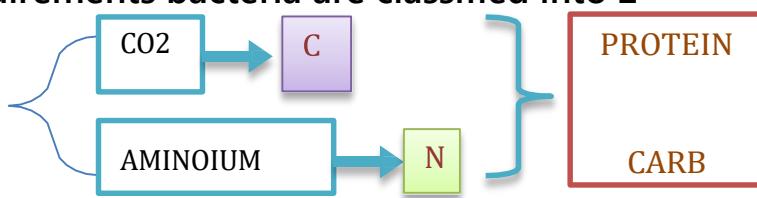


Bacterial Nutrition

According to nutritional requirements bacteria are classified into 2 main groups.

a-Autotrophic bacteria

b-Heterotrophic bacteria



Autotrophic bacteria	Heterotrophic bacteria
These are bacteria which can utilize simple inorganic substances	There are bacteria which require complex preformed organic substances
E.g. CO ₂ as a source of carbon, and ammonium salts as a source of nitrogen, from which they synthesize organic substances e.g.: proteins and carbohydrates	E.g. sugars, protein which are derived from plant or animal sources.
These are free living, non-parasitic (saprophytic) organisms of no medical importance	All bacteria of medical importance are heterotrophs

Growth factors

- These are **organic compounds** which a bacterial cell must contain in order to grow but is **unable to synthesize**
- e.g. amino acids, vitamins, purines, pyrimidines and pentose.
- These **essential compounds** have to be added.



Gaseous Requirements

- Oxygen requirements
- Carbon dioxide CO₂

A- Oxygen requirements

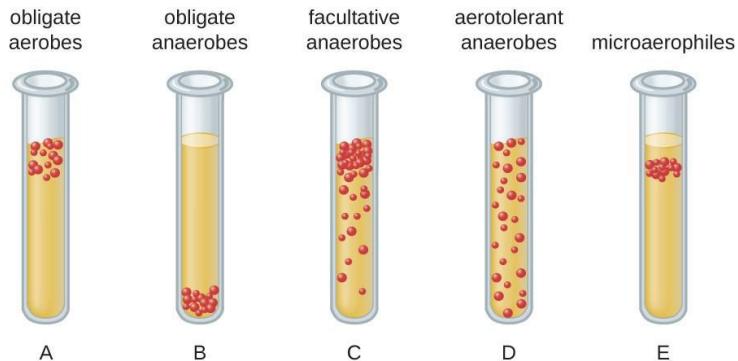
According to oxygen requirements bacteria are classified into 5 categories:

Obligatory aerobes	cannot grow except in presence of oxygen e.g. Mycobacterium tuberculosis and vibrio cholera
Obligatory anaerobes	Can grow only in complete absence of oxygen e.g. clostridia (Cl. tetani).
Facultative anaerobes	can grow in presence and in absence of oxygen e.g. staphylococci & E-coli. (most bacteria of medical importance)
Aerotolerant anaerobes	these have a fermentative (anaerobic) pattern of metabolism but can tolerate the presence of oxygen because they possess superoxide dismutase e.g. Cl. Perfringens
Microaerophilic	grow best in presence of minimum amount of oxygen e.g. campylobacter jejuni & corynebacterium acne

The basis of this classification

Aerobes and facultative anaerobes:

Contain certain enzymes (e.g. catalase, peroxidase and superoxide dismutase) which degrade these toxic compounds and protect the bacteria from their effect.



Anaerobic bacteria: Don't possess these protective enzymes and the presence of oxygen is harmful to them due to production of H₂O₂ and superoxide. Thus in anaerobic bacteria, the ultimate H₂ or electron acceptor are not O₂ but other organic or inorganic compounds.

B- CO₂

- Normal CO₂ concentration in atmospheric air (0.04%) is sufficient for growth of most bacteria.
- Some bacteria need high percentage (5 – 10%) of CO₂ e.g. Brucella abortus & Neisseria gonorrhoea
- CO₂ is needed to enhance toxin production by staphylococci and capsule formation by Bacillus anthracis. (Carbonophilic)



Moisture

- ✓ Good percentage (70%) of moist body weight of bacteria is water.
- ✓ High water content is essential for preparation of nutrient culture media for cultivation of bacteria. Staph. may survive dryness, this explain why staph. can spread easily by air. While Escherichia coli, Klebsiella and Pseudomonas don't.
- ✓ T.B cultures need high degree of moisture, so it grows in sealed tubes to avoid dehydration.

Temperature

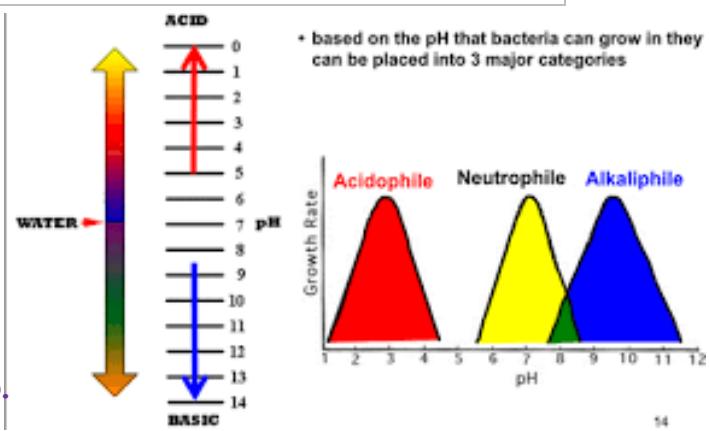
- ✓ Optimal temperature: It is the temperature at which growth of bacteria is optimal (most rapid). It is 37°C for pathogenic bacteria (Normal body temperature).
- ✓ temperature range is the range between minimum and maximum temperature
- ✓ Temperature 25 -40 °C is ideal for pathogenic bacteria (Mesophilic bacteria).
- ✓ Bacteria that can grow below the minimum temperature are called psychrophilic
- ✓ Bacteria that can grow above the maximum temperature are called thermophilic.

Classification based on Temp

Mesophilic	grows best between 25°C and 40°C. e.g. most bacterial pathogens
Psychrophilic (cold loving)	grows best below 20°C e.g. Flavobacterium spp
Thermophilic	grows best at high temp, 55-80°C e.g. Bacillus stearothermophilus

Hydrogen ion concentration (PH)

- ✓ Optimal pH: It is the pH at which growth of bacteria is optimal (most rapid),
- ✓ It is 7.5 for pathogenic bacteria, the same pH as body fluids
- ✓ PH range for pathogenic bacteria is 7.4- 7.6.
- ✓ Some bacteria tolerate alkaline media e.g. vibrio cholera.
- ✓ Some bacteria tolerate (prefer) acidic media e.g. lactobacillus



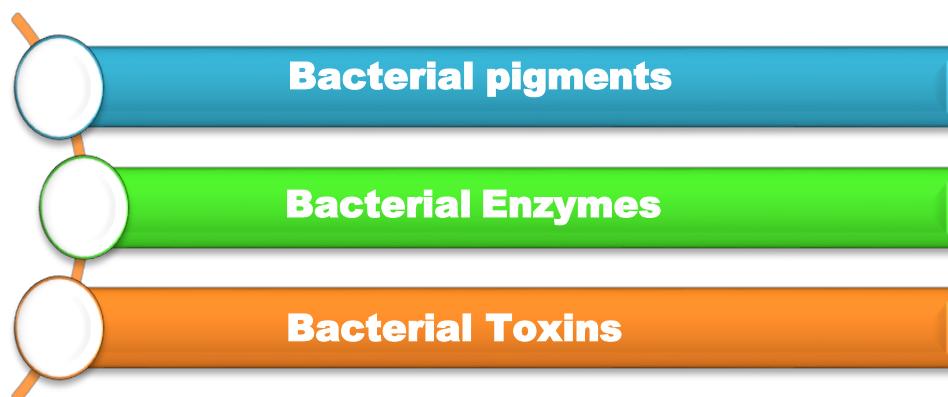


Light: Darkness provides a favorable condition for bacterial growth and viability

Osmotic pressure

Bacteria are tolerant to changes in the osmotic pressure of the environment, due to strong cell wall they have.

BACTERIAL PRODUCTS



Bacterial pigments

- ✓ These are colored substances produced by some bacteria.
- ✓ They are either endopigments or exopigments.

Endopigments	Exopigments
The pigments are non-diffusible (remain localized to body of bacteria).	The pigments are diffusible
Bacterial colonies are colored but the surrounding medium is not e.g.: Staph. aureus produce golden yellow colonies	Both bacterial colonies and the surrounding medium are colored e.g. Pseudomonas aeruginosa produce pyocyanin (blue) and fluorescent (yellow) pigments

N.B: -Bacterial pigments are best developed at room temperature on solid media, under aerobic conditions.

-Bacterial pigments play a role in respiration, some have anti-bacterial actions.





Bacterial Enzymes

- They are biological catalysts which mediate bacterial activities.
- They are protein in nature, and produced only by living cells.
- Enzymes are named according to the substance acted upon (substrate) by the enzyme e.g. proteolytic enzymes acts upon proteins, saccharolytic enzymes acts upon carbohydrates and lipolytic enzymes acts upon lipids.
- Dehydrogenases and oxidases are respiratory enzymes.
- The substrate: The substance which is acted upon by the enzyme.

Function of Bacterial Enzymes

- Metabolism e.g. lipase enzyme.
- Respiration e.g. cytochrome enzyme.
- DNA replication.
- DNA recombination.

Uses of Bacterial Enzymes

- Industry e.g. wine and cheese production.
- Laboratory e.g. restriction endonuclease enzyme in DNA - recombinant technology - gene therapy.
- Therapeutic e.g. streptokinase as a thrombolytic therapy in treatment of thrombosis

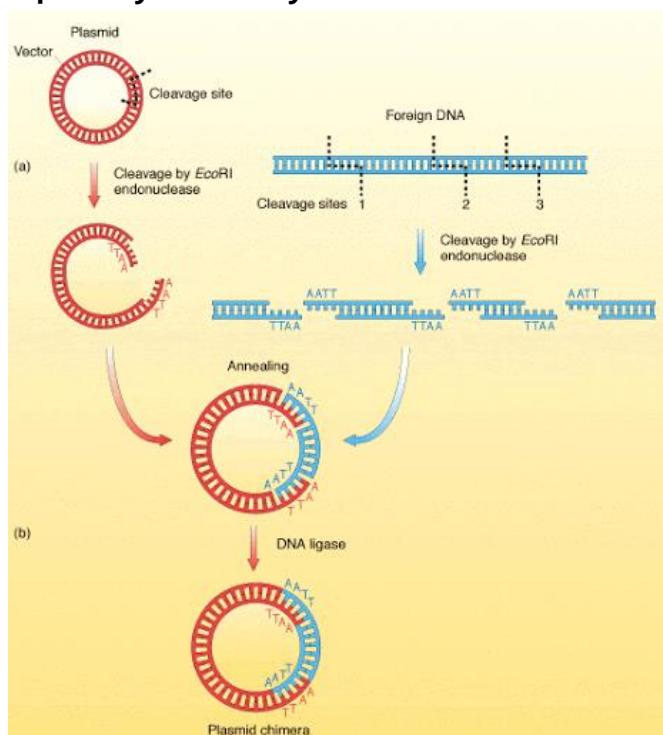
Bacterial Toxins

- These are poisonous substances produced by certain types of bacteria and are responsible for symptoms and signs of toxemia; they have inhibitory or lethal action on host cells.
- There are two types of toxins exotoxins and endotoxins

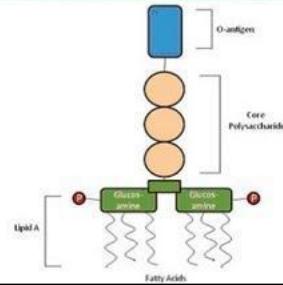
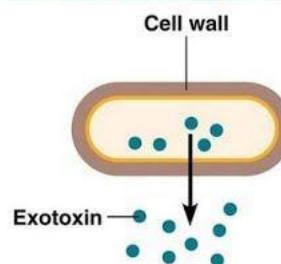
Differences Between Exotoxins and Endotoxins

(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.

(b) Endotoxin is the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.



EXOTOXINS VS ENDOTOXINS

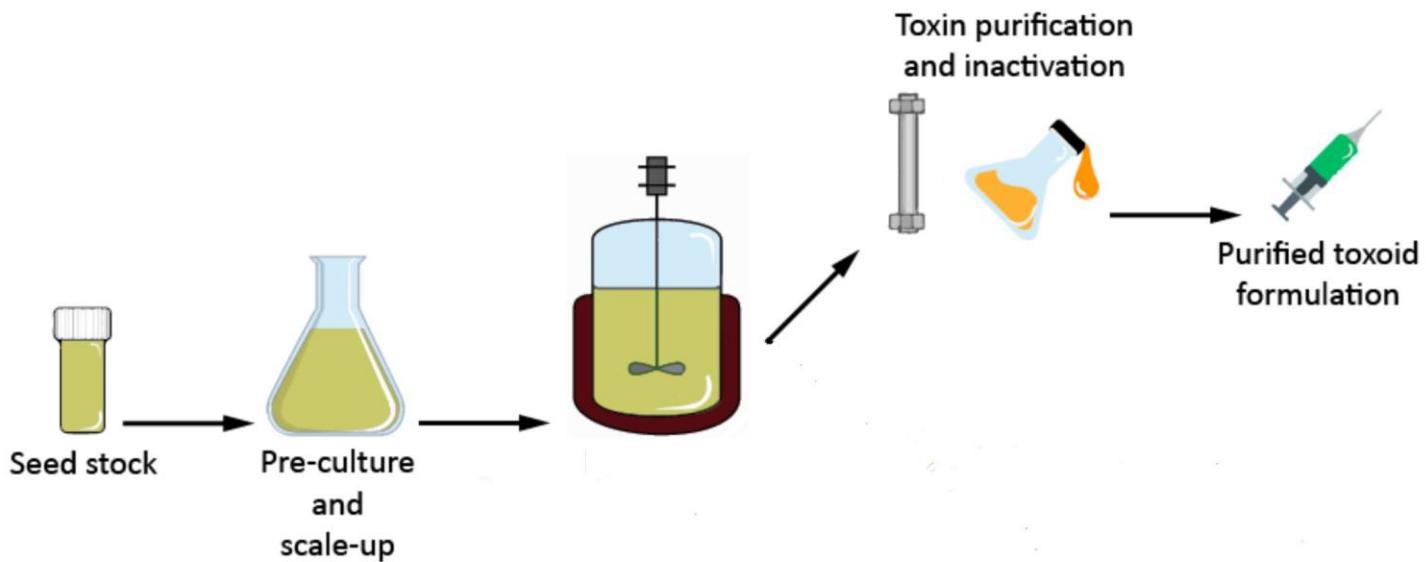




	Exotoxin	Endotoxin
Diffusibility	Diffusible	Non diffusible
Toxicity	Highly toxic	Less toxic
Antigenicity	Strong antigenic	Weak antigenic
Specificity	Specific in action	Non specific
Nature	Protein	Lipopolysaccharides
Effect of heat	Thermolabile	Thermostable
Effect of formalin	Detoxicated and changed into Toxoid	No effect
Production	Mainly by Gram +ve bacteria	Gram -ve bacteria
Location of genes	Plasmids (Extrachromosomal)	Chromosomes
Preparation	By filtration of broth culture of the organism.	By disintegration of the organism
Example	-Diphtheria exotoxin. -Cl. Tetani exotoxin	-Salmonella -Shigella

Uses of Exotoxin:

- Active immunization by formol toxoid.
- Passive immunization by antitoxic sera.
- Susceptibility test e.g. Schick's test in diphtheria and Dick's test in scarlet fever.





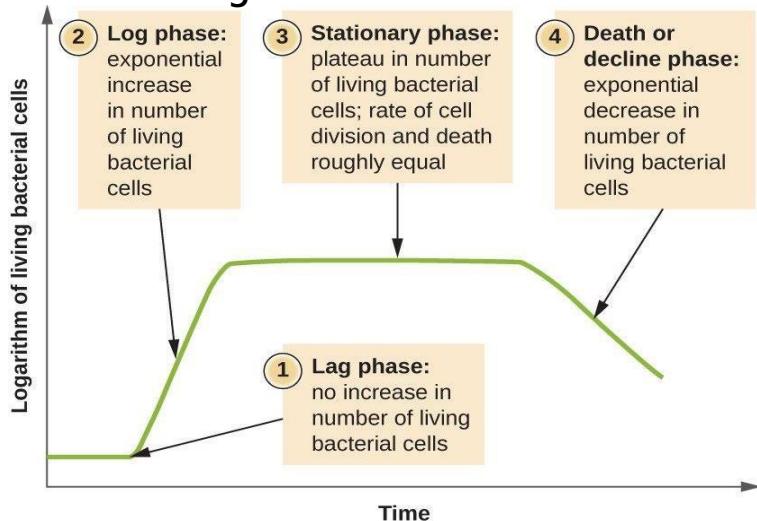
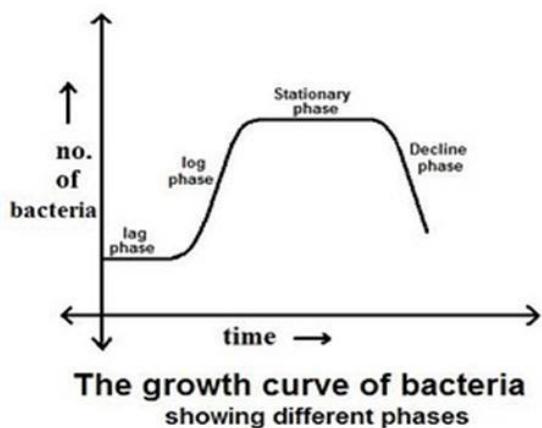
Antitoxins:

- Antitoxins are prepared from the blood of animals, usually horses, that have been immunized by repeated injections of specific bacterial exotoxins.
- The toxin, in constantly increasing doses, induces the formation of antitoxin in the blood of the injected animal.
- After tests have been conducted to determine the antitoxin titer of the serum, the animal is bled, the clot is permitted to form, and the clear supernatant serum is separated for processing.



Bacterial Reproduction and Growth Curve

When a given bacterium is inoculated on fluid medium, the growth is presented by plotting the logarithmic number of viable bacteria against time in hours.



1- Lag phase:

- During this phase the organism prepares itself to the 2nd stage of active division.
- No increase in the number of bacteria, but it is not a completely silent period since there is increase in size, and metabolic activity.
- The length of this phase varies from few hours to few days, it depends on the following factors:
 - 1) The organism: E. Coli has short lag phase, while T.B. has long lag phase.
 - 2) Size of inoculum: the bigger the size of the inoculum the shorter is the lag phase.
 - 3) The stage from which the inoculum is taken: the lag phase will be short if the inoculum is taken from logarithmic phase.
 - 4) The medium: the more suitable the medium, the shorter is the lag phase.



2- Logarithmic (exponential) phase:

- In this phase the division occurs at a maximum rate when the number of viable bacteria is increasing regularly (due to excess nutrients and oxygen – minimum waste products and CO₂).
- When the logarithm of number of viable bacteria is plotted against time in hours, a straight ascending line is produced.
- This stage will continue till one or more nutrients in the medium become exhausted or the toxic metabolic products accumulate.

3- Stationary phase:

- The rate of cell division slows down and the rate of death of cells increase till becomes equal. So the number of viable bacteria remains constant.
- **The decrease in growth rate is due to:**
 - a) Exhaustion of nutrients.
 - b) Exhaustion of O₂.
 - c) Accumulation of toxic materials.
 - d) Change in pH.

4- Decline phase:

- The rate of cell death is greater than cell division.
- This stage end in complete sterility of the medium (most bacteria are dead).
- This is due to accumulation of toxic waste products and exhaustion of nutrients and enzymes.

Clinical significance of growth curve:

The 4 phases of the bacterial growth curve correlate with the different stages of diseases as follow:

- **The lag phase corresponds** to incubation period.
- **The logarithmic and stationary phases** correspond to the symptoms and signs of the disease.
- **The decline phase** corresponds to convalescent stage.

However, in the animal body the course is modified according to the degree of virulence of the organism and the resistance of the host.



Data MCQ

1-Exotoxins are: a. Heat labile. b. Heat stable. c. Part of cell wall. d. Polymerized complexes.	5- In growth curve of bacteria, the number of bacterial cell divided is equal to that died in: a. Lag phase of growth curve of bacteria. b. Logarithmic phase of growth curve of bacteria. c. Stationary phase of growth curve of bacteria. d. Decline phase of growth curve of bacteria.
2-The bacterial cells are at their metabolic peak during: a. Lag phase. b. Logarithmic. c. Stationary. d. Decline.	6- Microaerophilic organisms: a. Grow only in the presence of O ₂ . b. Grow only in the absence of O ₂ . c. Grow only in the presence of small amount of O ₂ . d. Can grow either in the presence or the absence of O ₂ .
3-A facultative anaerobic is: a. Only grow anaerobically. b. Only grow in the presence of O ₂ . c. Ordinarily an anaerobe but can grow with O ₂ . d. Ordinarily an aerobe but can grow in absence of O ₂ .	7- One of the following is NOT a character of exotoxins: a. Diffuse outside bacterial cell. b. Specific in action. c. Strong antigenic. d. Prepared by disintegration of the organism.
4- Convalescence stage of the disease is corresponding to: a. Lag phase of growth curve of bacteria. b. Logarithmic phase of growth curve of bacteria. c. Stationary phase of growth curve of bacteria. d. Decline phase of growth curve of bacteria.	8- One of the following is NOT a character of endotoxins: a. Thermostable. b. Lipopolysaccharides. c. Changed into formol toxoid if incubated with 0.3 % formalin. d. Produced by Gram negative bacteria.



Data QUESTIONS

- **Compare between:**
 - ✓ anabolism and catabolism
 - ✓ oxidation and fermentation.
 - ✓ aerobic and anaerobic bacteria.
- **Define the following:**
 - ✓ mesophilic bacteria
 - ✓ autotrophic bacteria
- **Classify** Different types of bacteria according to nutrition and gaseous requirements.
- **Recognize** Bacterial products.
- **Explain** the bacterial growth and describe its clinical significance.

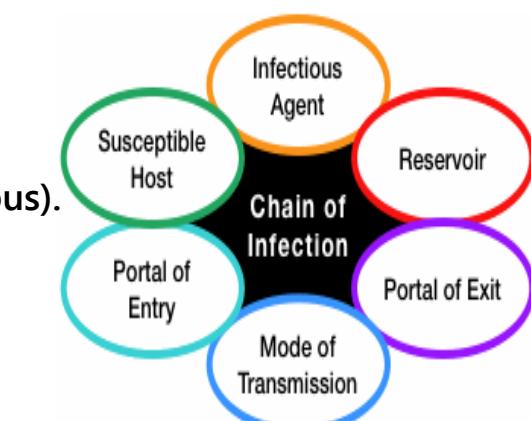
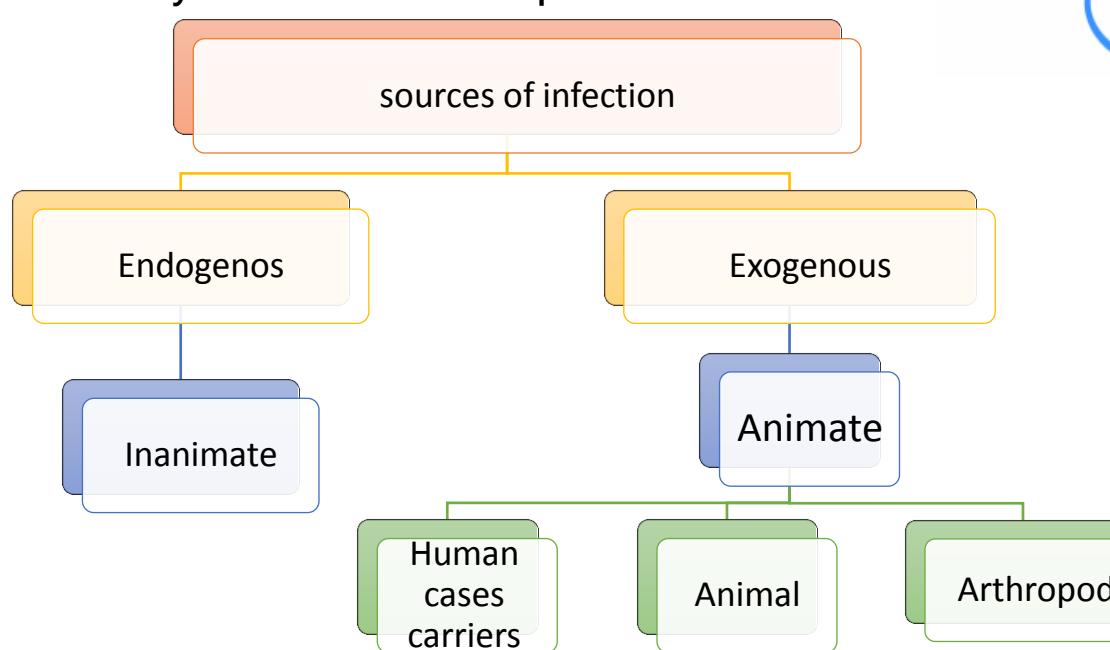


Micro L3 : Host- Parasite relationship

- Infection: Microorganism enters the body.
- Host: Infected body.
- Infectious disease: The disturbance in physiologic function of the host.

Infectious disease process (chain of infection):

- 1- Etiologic agent : (bacteria – virus – fungus).
- 2- Source of infection. = Reservoir (endogenous, exogenous).
- 3- Portal of exit.
- 4- Mode of transmission.
- 5- Portal of entry.
- 6- Susceptible host.



Endogenous source:

The **commensal (normal flora)** organisms normally existing in the body may **under certain condition** acquire **pathological** properties

- *E. coli* : (intestine) may produce urinary tract infection,
- *Strep. Viridans*: in throat (commensal) but if enter blood (SBE)
(subacute bacterial endocarditis)



Exogenous source:

Types of reservoirs:

- A. Human reservoirs: case, carrier
- B. Animal réservoirs: Zoonotic diseases
- C. Arthropode
- D. Environnemental réservoirs: soil

[A] Human (case):

- a) Typical case, showing typical signs and symptoms
- b) Subclinical case. the initial period of a disease when no symptoms or signs have yet manifested
- c) Latent case (latent infection).

[A] Human (carrier):

Carrier: A person who has the pathogenic organisms and passes them in his discharge (can infect others) **but** he is not diseased (no signs or symptoms).

Importance of carriers:

They are considered as a **dangerous source** of infection because:

- | | |
|---|--|
| 1-They are not easily detected. | 2-They are not known to public. |
| 3-They are not confined to bed | 4- Difficult to treat (explain). |
| 5- Reservoir of infection in inter-epidemic periods | |

Classification of carrier:

1] Site:

- | | |
|--------------------------------|----------------------------------|
| - Urinary carrier → Salmonella | -Intestinal carrier → Salmonella |
| -Throat carrier → Diphtheria. | -Nasal carrier → Staph |

[2] Duration: Transient or permanent (chronic)

[3] Mode of discharge: Intermittent or continuous



Portal of exit:

-Portal of exit is the path by which a pathogen leaves its host ex:

- a) Organisms of intestinal tract → **stools**.
- b) Respiratory organisms → **droplet or saliva**.
- c) Genital pathogens → **semen or cervical secretions**.

Modes of transmission:

Is the movement of pathogens from the reservoir to the host.

Types:

1- Vertical transmission:

From mother to her baby during pregnancy or labour.

2- Horizontal transmission:

-From one person to another.

-Contact, droplets ,air born, food born, blood born and arthropod born

Contact:

Direct contact: (hands to body surface)

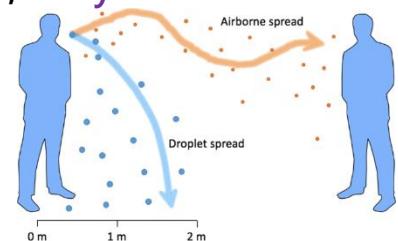


Indirect contact: (body surface or hands to a contaminated object e.g.

scissors, Foley catheters)

Droplet:

Particles $>5 \mu\text{m}$ in size may be generated through **coughing, sneezing, and suctioning**. Then they come into contact with the **mouth, nose, or eyes** of someone nearby.



Example: influenza

Airborne:

Airborne **droplet nuclei** $<5 \mu\text{m}$ in size are generated by coughing. Such droplet nuclei can **remain suspended in the air** for long periods of time.

Example: TB



Common vehicle:

Transmission by contaminated items such as food, water, medication, devices and equipment.

Vector borne:

Vectors, such as mosquitoes, may transmit infections.

Portal of entry:

-Is the path by which a pathogen enters a susceptible host.

Through non intact skin, respiratory tract or mouth.

Susceptible host:

-A person lacking effective resistance to a Particular pathogenic agent

MCQis A person or animal that harbors a specific infectious agent without discernible clinical disease and serves as a potential source of infection

- A- typical case
- B- latent case
- C- carrier
- D- sub clinical case

Factors that control disease production:

[1] Microbial factors : (Pathogenicity & virulence)

[2] Host factors : (Natural & Acquired immunity)

- Pathogenicity: Ability of the organism to produce a disease.
- Virulence: Degree of pathogenicity

Measurement of virulence:

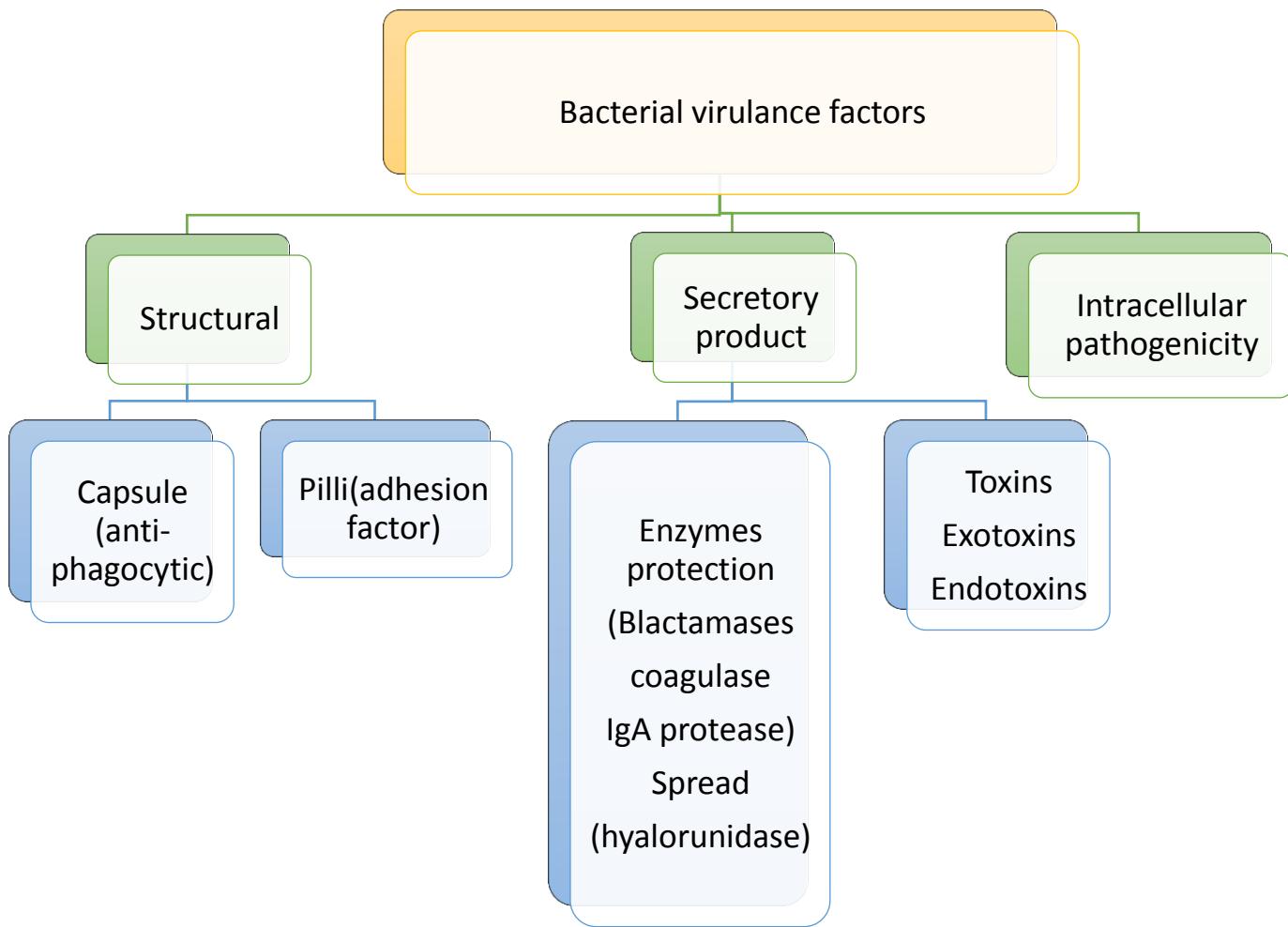
1- Minimal lethal dose :

The minimal dose of the organism (or toxin) that kill 100% (all) susceptible animals in a standard period of time.



2- Lethal dose 50 :

The **minimal dose** of the organism (or toxin) that **kill 50%** of susceptible animals in a standard period of time



Virulence factors:

1- Capsule: - Anti phagocytic (prevent phagocytosis).

- Made of polysaccharides (e.g. Pneumococcus) except Anthrax bacillus (protein).

2- Pili: Organs of adhesions (e.g. gonococcus)

3- Exotoxins

4- Endotoxins: Compare ?



5- Enzymes:

Coagulase – Hyaluronidase – B lactamase – IgA protease

- Coagulase (Staph) : changes fibrinogen \rightarrow fibrin which surround staph, prevent phagocytosis.
- Hyaluronidase (Strept) : dissolve hyaluronic acid help spread of infection
- B lactamase (penicillinase) : destroy B lactam ring of penicillin
- IgA protease : degrade IgA on mucus membranes (pnumococcus)

6- Intracellular habitat: T.B.



Micro L4 : Bacterial genetics

- **Gene:**

- is the **functional unit** of inheritance,
- it a segment of **DNA** that carries **information** for a specific function (control **specific property**).

NB:- Bacterial genes are found in : chromosome , plasmids , transposons & bacteriophage

1-chromosome:

- Bacteria are **haploid** (have single chromosome).
- A single DS DNA molecule which is coiled on it-self to form nuclear mass.
- It is a self replicating genetic element (**replicon**)
- **Function:**
 - 1- Carry genetic information.
 - 2- Help in cell division (chromosome replication).
 - 3- Protein synthesis.

N.B: Genetic code: it is the sequence of purine and pyrimidine bases in DNA molecule which control the production of amino acids (protein).

Protein synthesis (gene expression):

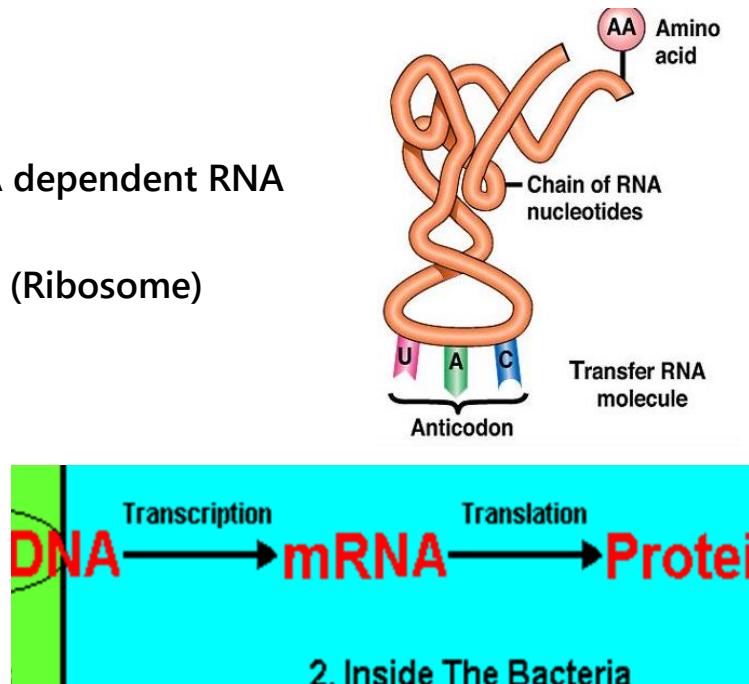
- **Transcription:** (nucleus)
- Formation of mRNA from DNA ,by DNA dependent RNA polymerase.
- Then mRNA leave nucleus to cytoplasm (Ribosome)
- Each 3 bases on mRNA = codon.

Amino acid activation:

- The amino acid is activated.
- tRNA in cytoplasm carry activated amino acid at one end and anticodon on other end.

Translation:

- Formation of protein by Ribosomes according to **base sequence** in mRNA.
- **Anticodon (t RNA)** unite with **codon (m RNA)** and an amino acid is introduced.



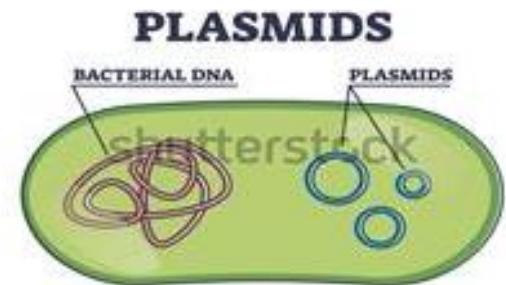


- Protein: (polypeptide is formed by linking amino acids together).
- **NB:** The 3 types of RNA are: mRNA , tRNA & rRNA.

2-Plasmid:

Def.: Extrachromosomal genetic elements

- They are circular coiled DS DNA molecule
- They are self replicating (Replicon).



Compare plasmid with chromosome:

- . Similar in: Circular, Coiled, DS, DNA, Replicon (self replicating)
- . Differ in: Smaller, Carry small number of genes , Single or multiple
- . Plasmids code for properties not essential for life Extrachromosomal

Bacterial chromosome	Plasmid
Circular ,Coiled	Circular ,Coiled
Double stranded DNA	Double stranded DNA
Replicon (self replicating)	Replicon (self replicating)
Larger – carry larger number of genes	Smaller - Carry small number of genes
Single	Single or multiple
code for properties essential for life: cell wall peptidoglycan	code for properties not essential for life: drug resistance, toxin production

Medical importance:

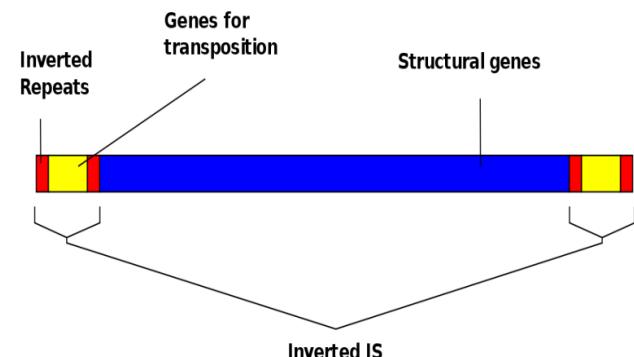
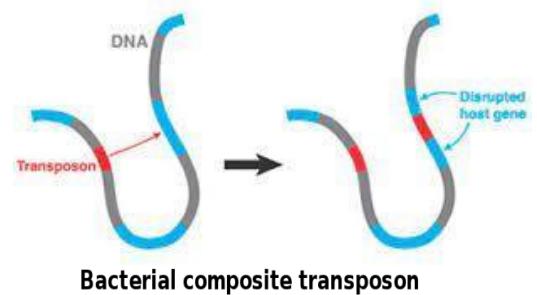
- . **R Factor:** R plasmid = Drug resistance plasmid (plasmid carry genes for drug resistance)
- . **F factor:** fertility factor = sex plasmid : Conjugative plasmids plasmid carry genes for pilus formation
- . **Virulence plasmids:** carry genes for production of enterotoxin, exotoxins.
- . **Plasmids for protective functions:** carry genes for resistance to ultra violet rays (UVR) and certain phages.

Transposons = Jumping genes:

Def.: These are segments of DNA that can move from one site in DNA molecule to another site in the same or different DNA molecule.



- Properties:**
- No autonomous replication.
- Have (**inverted repeats**) = special sequence at each end needed for insertion. ie : AACCAA or GGTTGG
- Have genes for production of enzyme needed for integration
- It may carry drug resistance gene (structural gene)
- Insertion of transposon into a gene affect its function (mutation)



Bacteriophages (Bacterial viruses):

Def.: are viruses that replicate as obligate intracellular parasites in bacteria

Morphology= (structure):

1. Head:

- Nucleic acid core (DNA or RNA) which is infectious part of the virus

- Capsid (protein) for the Protection of nucleic acid

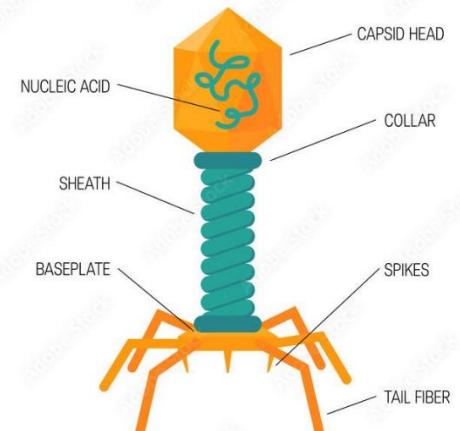
2. Tail:

- Hollow tube: through which pass nucleic acid.

- contractile sheath= tail sheath when contract bring head (NA) near to the hostcell.

- Base plate: carry tail fibers

- Tail fibers: for the attachment to host cells (bacteria)



STRUCTURE OF BACTERIOPHAGE

Classification: Phages are classified into 2 major groups:

virulent and temperate.

- Growth of virulent phages in susceptible bacteria destroys the host cells (lytic infection).
- Infection of susceptible bacteria by temperate phages leads to integration of viral genome into host DNA as a prophage without killing it (lysogeny) . Under certain circumstances, it may start lytic infection.

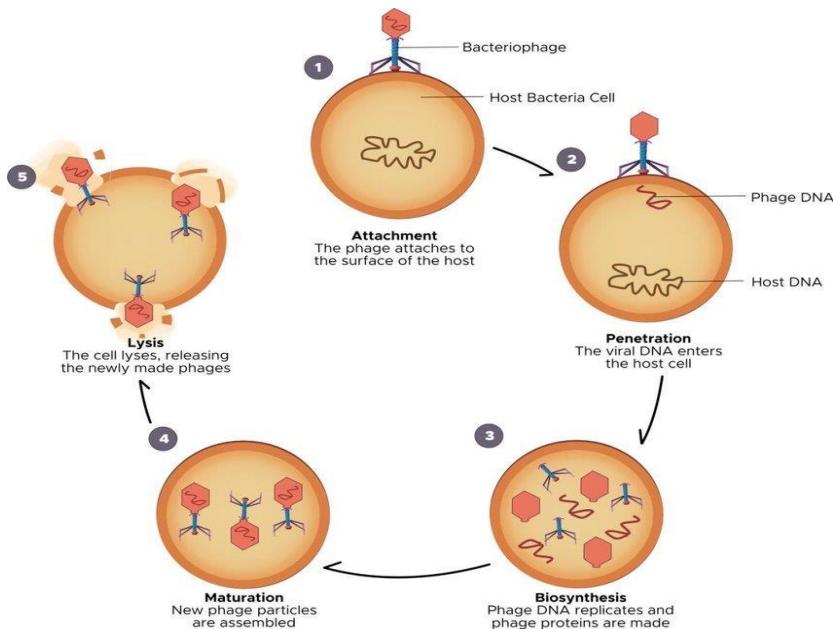


Replication of bacteriophage: 2 cycles for replication.

- 1- lytic cycle.
- 2- Lysogenic cycle.

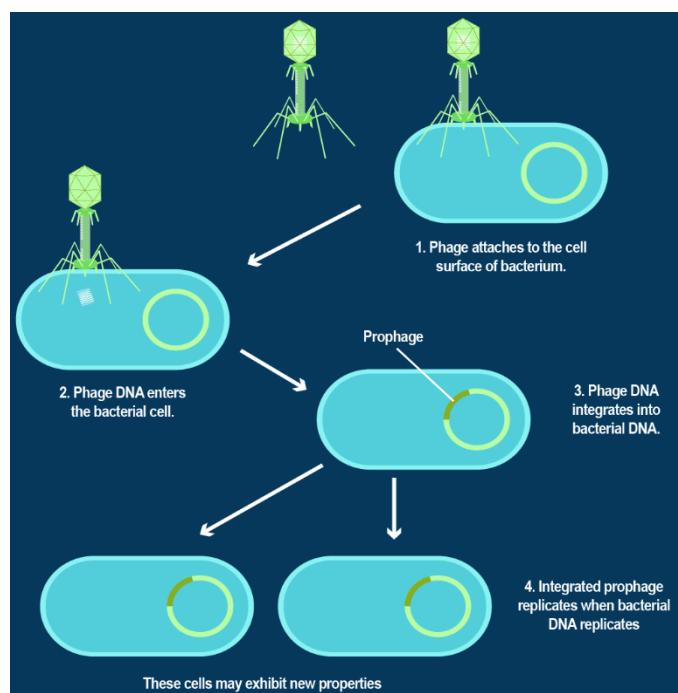
Lytic cycle:

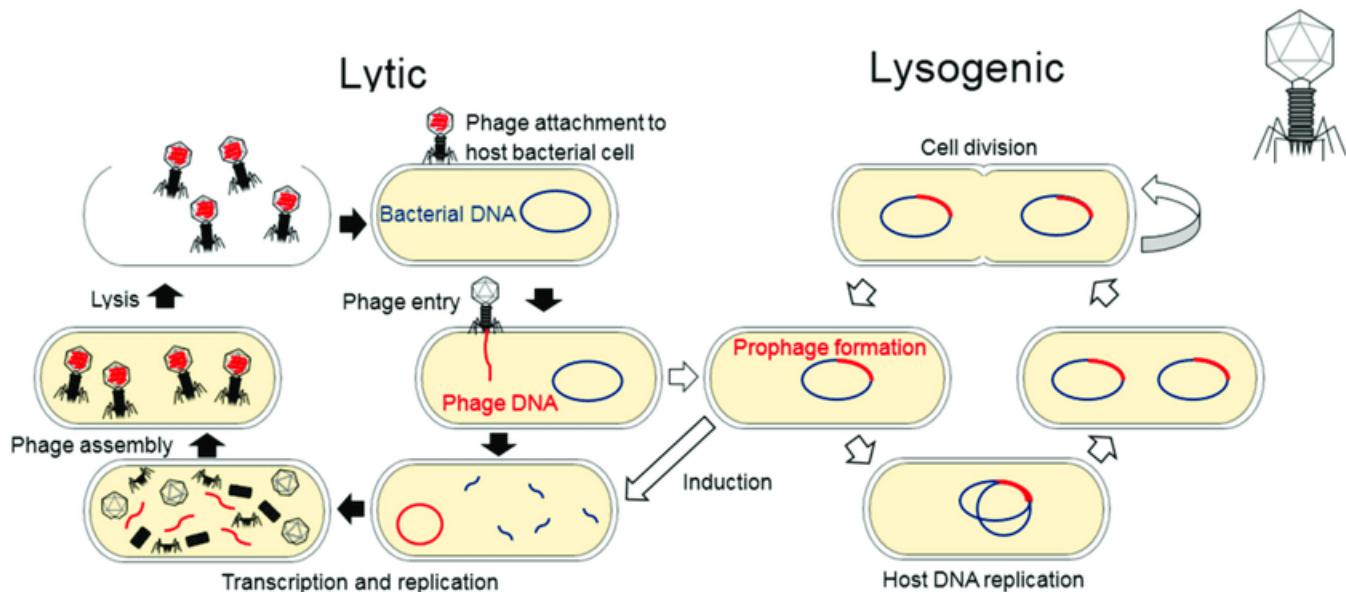
1. Adsorption: Tail fibers are attached to receptors on the bacterial cell.
2. Penetration: Nucleic acid of the phage enters the bacterial cell.
3. Un coating: Protein capsid remains outside the bacterial cell.
4. Eclipse phase: Intra cellular phage inside bacterial cell can not be detected.
5. Intra cellular synthesis: Synthesis of viral NA & viral proteins.
6. Assembly: Viral NA + Viral capsid protein = New phage
7. Viral release: Lysis of bacterial cell, and release of new phages
 - The cycle ends by:
 - lysis of host cells
 - and release of new viruses to infect other cells.



Lysogenic cycle:

- Lysogeny: It is the process by which phage DNA is incorporated into bacterial chromosome
- Prophage: Phage DNA integrated into bacterial cell chromosome\
- Lysogenic bacteria: Bacteria which contain prophage





Outcome of temperate phage cycle:

1. The prophage passes to daughter cells.
2. The prophage may be induced (detached from bacterial chromosome) → lytic cycle
3. **Specialized transduction.**
 - As the prophage excises from the bacterial genome, it may carry with it part of the adjacent genetic material of the bacterial chromosome.
 - As it infects another bacterium, it will transmit to it new characters. This is called specialized transduction

MCQ: R plasmids are the plasmids that:

- a) Influence bacterial toxin production
- b) Confer antibiotic resistance
- c) Regulate the structural components of pathogenic bacteria
- d) None of the above

Bacterial variation:

Def.: Change in properties of bacterial cells, may be phenotypic or genotypic

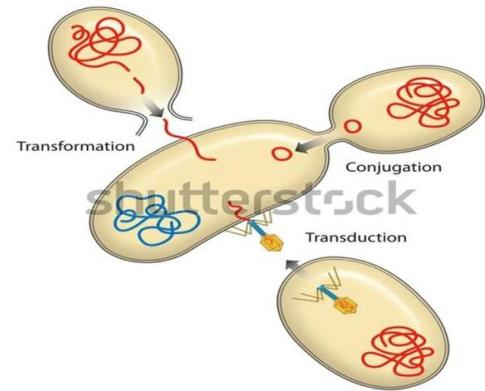
- 1) **Phenotypic variation:** are changes in the bacterial characters under the influence of the environment with no underlying genetic change.
 - They are Reversible and Not heritable
 - E.g.: Loss of flagella, ↑ Pigment production at room temperature
- 2) **Genotypic variation:** due to change in nucleotide sequence of the DNA.
 - They are Irreversible and heritable
 - E.g.: - Mutation, Gene transfer



Gene transfer:

Def.: Transfer of DNA from one cell to another. There are three types of gene transfer:

- 1. Transformation
- 2. Transduction
- 3. Conjugation



Competent cell:

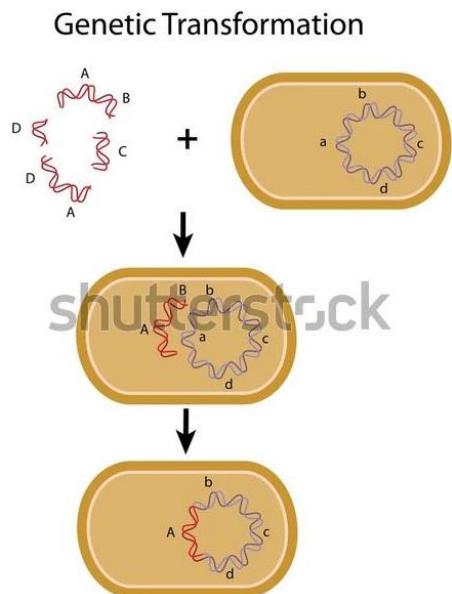
The recipient cell when it is able to take DNA from and incorporate it in its DNA.

[1] Transformation:

Def.: the transfer of naked DNA (released from the donor cell to the medium) to another cell.

Mechanism:

- At first, the donor bacterial cell is lysed, and its DNA is released in the environment
- Then the recipient cell takes it from the surrounding medium.
- The recipient cell must be in a state of "competence" to uptake the donor DNA.



[2] Transduction:

Def. It is the process by which DNA fragment is transferred from one bacterium to another by means of a virus (bacteriophage).

It is of 2 types:

- Generalized transduction.
- Specialized transduction.

Generalized transduction:

- Transduction in which the phage can carry any part of donor DNA.
- It occurs in lytic cycle
- virulent phage



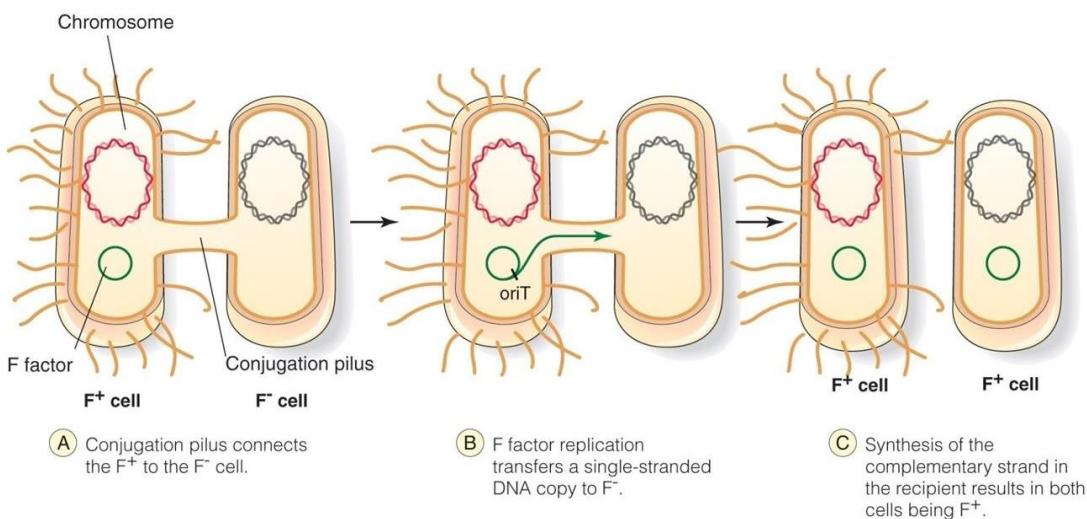
Specialized transduction:

- Transduction in which the phage will carry the part of DNA adjacent to its insertion in bacterial chromosome (carry special part).
- Occur in lysogenic cycle. - Temperate phage

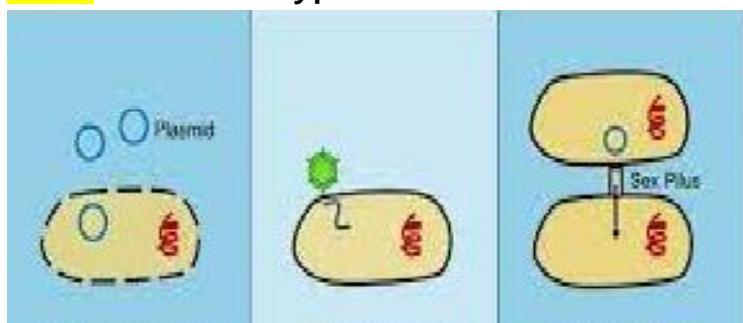
[3] Conjugation:

Def.: direct contact between the donor (male) cell and the recipient (female) cell, leads to establishment of a **cytoplasmic bridge** between both cells, and transfer of part or whole genome to the recipient cell.

- The donor cell has fertility plasmid (F plasmid) or sex plasmid which gives it the ability to form cytoplasmic bridge with the recipient cell.
- The donor cell is called F⁺ cell.
- One strand of donor DNA passes from F⁺ to F⁻ cell.
- A complementary strand is formed to form a double stranded F factor plasmid in both the donor and the recipient cells
- So after conjugation the donor cell remains F⁺ while the recipient cell changes from F⁻ to F⁺ cells



Quiz: Name the type of Gene transfer?





Gene therapy:

Def:- It is a technique that uses genes to prevent or cure a disease or medical disorder (replacement of a defective gene by a normal one).

Types:

- **Ex vivo** gene therapy involves the genetic modification of cells outside of the body to produce therapeutic factors and their subsequent transplantation back into patients.
- **In vivo** gene therapy uses viruses or other methods to deliver genes directly into your cells.

Gene delivery methods:

a) Viral gene delivery: e.g. Adeno V.

- Advantages:
- Ability to bind to cell and enter inside.
- Avoid Intracellular degradation.
- The gene reach the nuclear.

b) Non viral gene delivery:

- Liposomes: consist of lipid bilayer which have high affinity for cell membrane.
- DNA-ligand conjugates: DNA is complexed to a ligand that binds to cell receptors.
- Naked DNA: the use of purified DNA in the form of plasmid.

Uses:

- 1- Treatment of genetic diseases.
- 2- Treatment of I.D. Diseases.
- 3- Treatment of tumor



Micro L5 : Antimicrobials

Antimicrobials

Mechanism of action of drugs

Type	Inhibition of cell wall synthesis	Inhibition of cell membrane functions	Inhibition of protein synthesis	Inhibition of nucleic acid synthesis
Example	<ul style="list-style-type: none"> ❖ Penicillin ❖ Cephalosporin ❖ Vancomycin ❖ Bacitracin 	<ul style="list-style-type: none"> ❖ Amphotericin B ❖ Colistin ❖ Polymyxin 	<ul style="list-style-type: none"> ❖ Chloramphenicol ❖ Erythromycin ❖ Aminoglycosides ❖ Tetracyclines 	<ul style="list-style-type: none"> ❖ Quinolones ❖ Nalidixic acid ❖ Novobiocin ❖ Rifampicin ❖ Sulfonamides
Mechanism	<p>All B-lactam drugs are selective inhibitors to cell wall synthesis:</p> <ul style="list-style-type: none"> ❖ 1st bind to cell receptors (Penicillin binding receptors (PBPs), They under chromosomal control) ❖ Removal or inactivation of inhibitors of autolytic enzymes in the cell wall ❖ Inhibition of transpeptidation reaction enzymes (Loss of D-alanine from peptidoglycan) ❖ This will lead to lyses of the cell 	<ul style="list-style-type: none"> ❖ The cytoplasmic membrane controls internal components of the cell. ❖ If cytoplasmic m. damaged macromolecules and ions escape from the cell leading to cell damage and death. 	<p>1) By acting on 30 s ribosomes:</p> <ul style="list-style-type: none"> ❖ Aminoglycosides ❖ Tetracyclines <p>2) By acting on 50 s ribosomes:</p> <ul style="list-style-type: none"> ❖ Chloramphenicol ❖ Macrolides ❖ Lincomycins 	
Note	<ul style="list-style-type: none"> ❖ Resistance to penicillin may be determined by the production of B-lactamases by organisms ❖ Some B-lactamases are plasmid mediated and others are chromosomal mediate ❖ Polymyxins: Acting on Gm –ve bacteria ❖ Polyenes: Acting on fungi ,which need to bind to sterol found in C.M. of fungi and not present in bacterial C.M 			

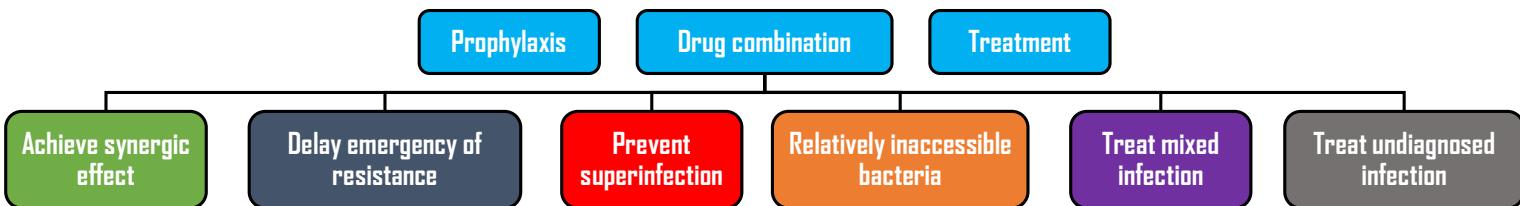




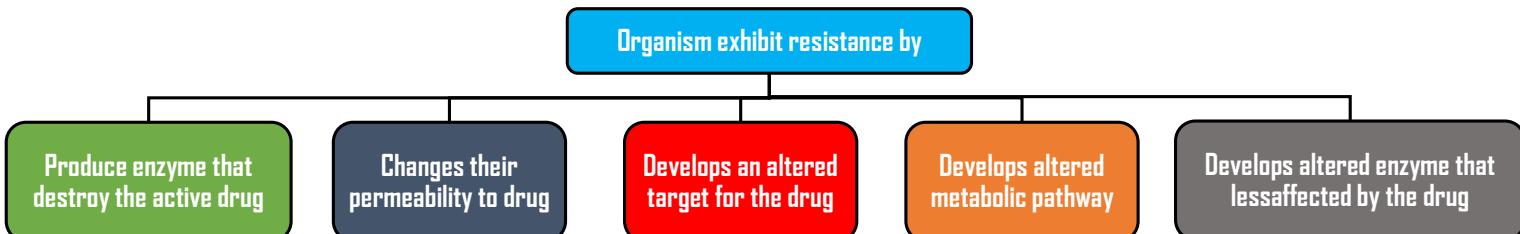
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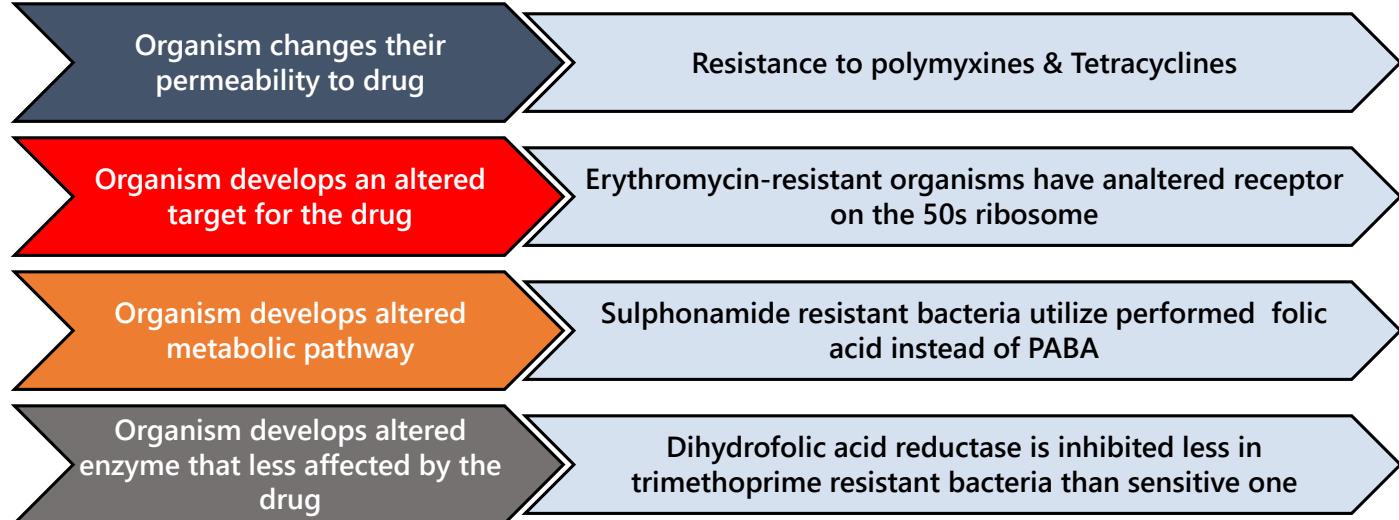
Selective toxicity	Synergism	Antagonism
<ul style="list-style-type: none"> ❖ The drug is harmful to a pathogen but not harmful to the host . ❖ It is relative than absolute 	<ul style="list-style-type: none"> ❖ the action of two drugs is more than the sum of the two drugs 	<ul style="list-style-type: none"> ❖ One drug antagonizes the action of other drug when given simultaneously
Examples		
<ol style="list-style-type: none"> 1) When bacteriostatic drug (tetracycline and chloramphenicol) used with bactericidal one (aminoglycoside and penicillin) the bacteriostatic reach first to the infection site . 2) Use B-lactam drugs as imipenem and piperacillin if used in treatment of <i>P. aerogenosa</i> as imipenem is a potent inducer of B-lactamase 		

Uses of antibacterial drugs



Resistance of antibacterial drugs





Origin of Drug resistance

Nongenetic origin	Genetic origin
<ul style="list-style-type: none"> ❖ Inert organism ❖ Loss of cell wall ❖ Bacteria in site not reached by drug 	<p>1) Chromosomal</p> <ul style="list-style-type: none"> ❖ Spontaneous mutation change receptors of drug . ❖ Loss of PBP. of penicillin . <p>2) Extra chromosomal e.g. plasmid</p> <ul style="list-style-type: none"> ❖ This Can be transferred by <ol style="list-style-type: none"> 1. Transformation 2. Transduction 3. Conjugation

Factors affecting antimicrobial drugs

- ❖ PH of the environment .
- ❖ Component of medium .
- ❖ Stability of drug .
- ❖ Size of inoculum
- ❖ Length of incubation .
- ❖ Metabolic activity of microorganism





Drug dependence	Cross- resistance
<ul style="list-style-type: none"> ❖ Certain organisms are not resistant to a drug but require it for their growth. ❖ As streptomycin - dependent meningococci in mice 	<ul style="list-style-type: none"> ❖ Microorganisms resistant to a certain drug also be resistant to other drugs that share a mechanism of action.

limitation of drug resistance

- ❖ Maintain sufficient high level of drug .
- ❖ Simultaneously administration of 2 drugs that do not give cross-resistance .
- ❖ Restriction the use of drug especially in hospitals .

Measurement of antimicrobial activity

- 1) Dilution test
- 2) Diffusion method
- 3) Automation

Indiscriminate use of drug

- 1) Sensitization of patients (hypersensitivity ,anaphylaxis , rashes , blood disorder)
- 2) Change in the normal flora of the body .
- 3) Masking serious infection .
- 4) Direct drug toxicity .
- 5) Development of drug resistance .
- 6) Affecting immune response

Antituberculosis Drugs

1 st line treatment	2 nd line treatment (for resistant strains to 1 st line)
<ul style="list-style-type: none"> ❖ Streptomycin ❖ Isoniazid ❖ Ethambutol ❖ Rifampicin 	<ul style="list-style-type: none"> ❖ Kanamycin ❖ Para-amino-salicylic acid ❖ Cycloserine ❖ Ciprofloxacin





Anti-Viral Drugs

Type	Nucleoside analog	Nucleotide analogs	Nonnucleoside reverse transcriptase inhibitors	Protease inhibitors	Fusion inhibitors	Other types
Example	<ul style="list-style-type: none"> ❖ Acyclovir ❖ Lamivudine ❖ Ribavirin ❖ Vidarabin ❖ Zidovudine 	<ul style="list-style-type: none"> ❖ Cidofovir 	<ul style="list-style-type: none"> ❖ Nevirapine 	<ul style="list-style-type: none"> ❖ Saquinavir 	<ul style="list-style-type: none"> ❖ Fuzeon 	<ul style="list-style-type: none"> ❖ Amantadine ❖ Rimantadine ❖ Interferons
Mechanism	<ul style="list-style-type: none"> ❖ They inhibit nucleic acid replication 		<ul style="list-style-type: none"> ❖ Acting by binding directly to reverse transcriptase 	<ul style="list-style-type: none"> ❖ Inhibition of protease yields non-infectious viral particles 	<ul style="list-style-type: none"> ❖ Blocks the virus and cellular membrane fusion yielding non-infectious viral particles 	-





Micro L6 : General virology

GENERAL PROPERTIES OF VIRUSES:

- Viruses are the smallest known infective obligate intracellular agents.
- They infect most forms of life (human, animal, plants and bacteria).
- They are very small {Their size ranged from 10- 300nm therefore} :
 - They are seen only by the electron microscope (except poxviruses).
 - They can pass through the bacterial filters.
 - Require ultracentrifugation for their centrifugation (10,000-30,000 rpm).
- Their genome is either DNA or RNA, single stranded or double stranded
- They have a receptor-binding site that reacts specifically with a corresponding receptor located on the surface of the infected cell (key and lock)
- All viruses are metabolically inert as they have no activity outside the host cell.
- They are obligatory intracellular parasites as they are totally dependent upon a living cell for replication and existence

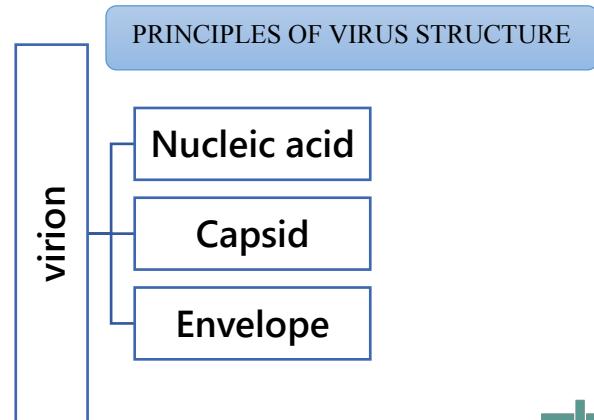
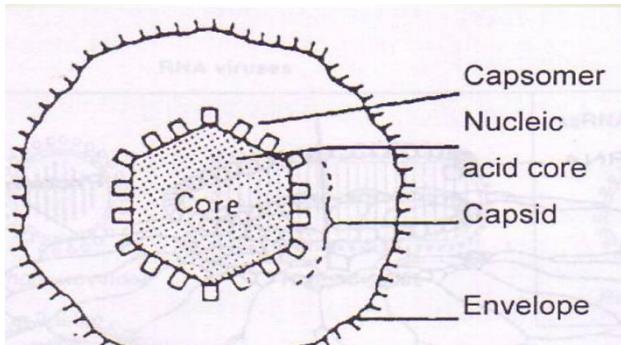
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because viruses lack:

- 1) ATP generating system.
- 2) Structure sites of protein synthesis i.e. ribosomes and protein synthesizing apparatus

this explains that :

- Viruses are not able to multiply in inanimate media but only inside living cells.
- They are not susceptible to antibiotics or any agents acting on the metabolic pathways.
- Most viruses are sensitive to interferon
- The viruses reproduced by special replication cycle.
- Some viruses can induce latent infection by integration of their nucleic acid with the DNA of the infected cells



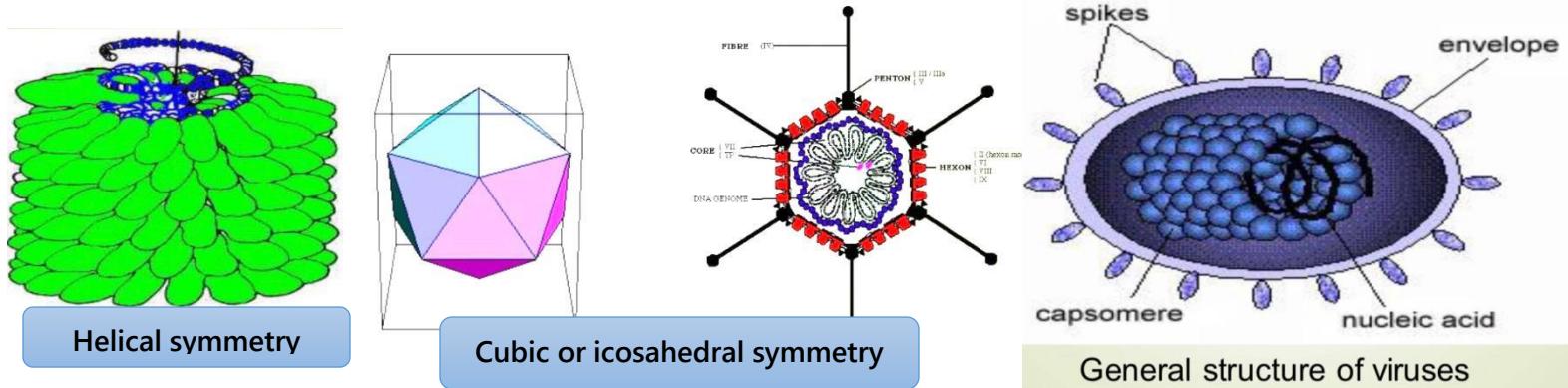


Nucleic acid (viral genome)	<ul style="list-style-type: none"> • It is either DNA or RNA intact or segmented, linear or circular. • Accordingly, viruses are classified into either DNA or RNA viruses. • Each nucleic acid may be present as a single stranded or double stranded <p>⇒ Functions of nucleic acid:</p> <ul style="list-style-type: none"> - It is the essential infectious component of the virus. - It carries the heritable characters of the virus. - It codes for the production of new viral proteins and genomes in viral replication
Capsid (viral protein)	<ul style="list-style-type: none"> • It is a protein coat surrounding the nucleic acid. • It is composed of 32- 250 protein structural units called capsomeres. • The capsid with its enclosed nucleic acid is called nucleocapsid. <p>⇒ Functions of Capsid:</p> <ul style="list-style-type: none"> - Protection of nucleic acid core from both physical destruction and enzymatic hydrolysis by nuclease enzymes (present in the serum) - The number of capsomeres is used in classification and identification of viruses. - The symmetry of capsomeres is responsible for the ultimate shape of the viruses - It possesses receptor binding sites that enable the virus to adsorb or attach to a specific receptor site on the host cell. - Viral proteins are antigenic and immunogenic.
Envelope	<p>Many virions are surrounded by a lipoprotein envelope that was acquired during their final stage of replication when the virus particles bud through the host cell membranes</p> <p>⇒ Functions of Envelope:</p> <ul style="list-style-type: none"> - Attachment to the host cell receptors - Attachment of its antigen to RBCs leading to haemagglutination . - May have an enzymatic activity. - May induce a foreign viral antigen on the infected host cell. - e-This envelope renders the virus sensitive to bile salts and lipid solvents e.g., ether and chloroform



❖ shape of the viruses according to symmetry of capsomeres:

- **Helical Symmetry**: It is found in most RNA viruses
- **Cubic (icosahedral or isometric symmetry)** : It is found in all DNA viruses except poxvirus.
- **Complex symmetry** : As in poxviruses.



Virion: Is a complete infective viral particle i.e. nucleocapsid with or without envelope.

Viroid: Naked nucleic acid molecule without protein coat. It is infectious agent of plants (not found in humans or animals).

❖ EFFECT OF PHYSICAL AND CHEMICAL AGENTS ON VIRUSES:

1) Temperature:

- Heat : Most viruses are inactivated if heated at 56°C for 30 minutes or at 100 °C for few seconds.
- Autoclaving (121 °C for 15 mins) inactivates all types of viruses.
- Cold : Viruses are usually preserved at – 35 to -70 °C and by lyophilization (drying from the frozen state at high vacuum).
- Some viruses are partially inactivated by the process of freezing and thawing.

2) Drying : Some viruses are inactivated, and some survive.

3) Ultra-violet irradiation : All types of radiation inactivates viruses through its effect on viral nucleic acid.

4) Chloroform, bile and ether : Inactivate enveloped viruses.

5) Oxidizing agents : viruses are inactivated by formaldehyde, chlorine, iodine, and hydrogen peroxide.



6) Detergents and phenol : Most viruses are relatively resistant.

7) Glycerol :

- 50% kill the bacteria and preserve virus.
- It is used as a preservative to prevent bacterial contamination of viral suspensions.

8) Salts :

- Molar concentration of salts can stabilize viruses (i.e. not inactivated even by heating at 50 oC for one hour) e.g. molar MgCl used for stabilization of polio vaccine.
- This stabilized polio vaccine can keep its potency at room temperature while non stabilized polio vaccine must be stored in freezing state to preserve its potency.

9) Vital dyes :

- Viruses are penetrable to a varying neutral red and gentian violet. degree by toluidine blue
- These dyes bind to the viral nucleic acid which becomes susceptible to inactivation by visible light as in the treatment of skin lesion of herpes.

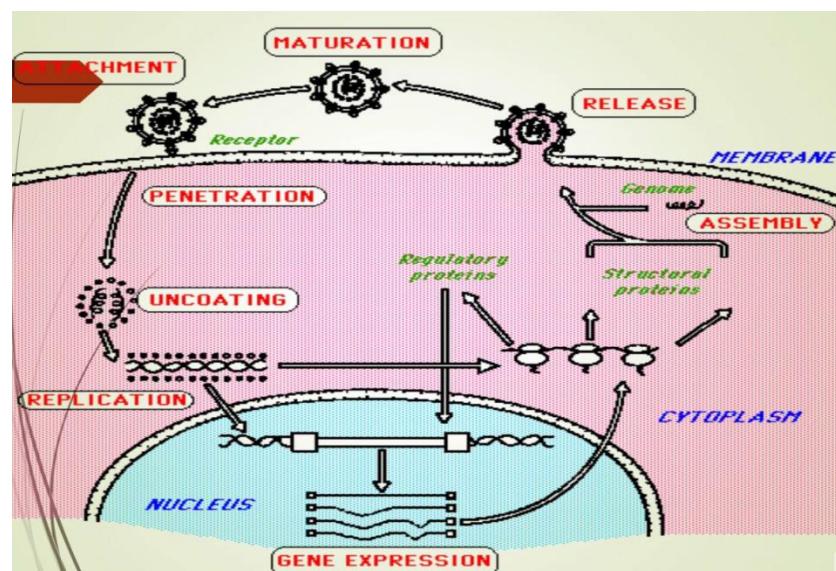
10) Bactericidal agents :

- The most effective disinfectants against viruses are oxidizing agents e.g. H₂O₂ and K permanganate.
- Formalin is used to disinfect many viral vaccine (not affect viruses themselves).
- Alcohol (methy1 or ethy1) in conc. of 70% inactivate most viruses

❖Viral replication

Steps in Viral Replication

- * Adsorption
- * Penetration
- * Uncoating
- * Eclipse phase
- * Viral genome replication
- * Assembly
- * Release



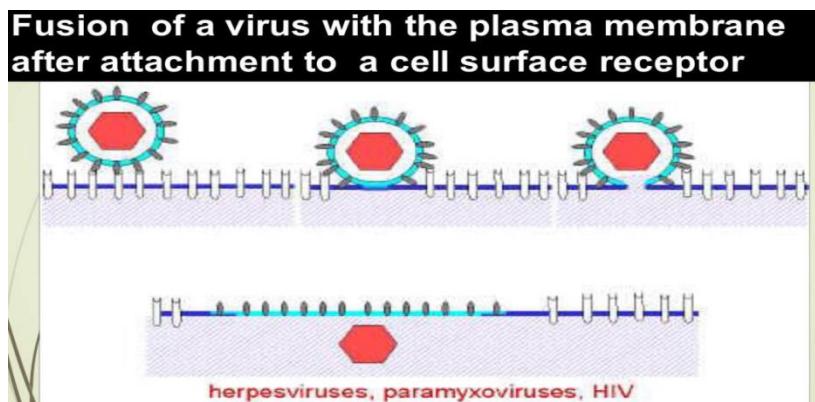
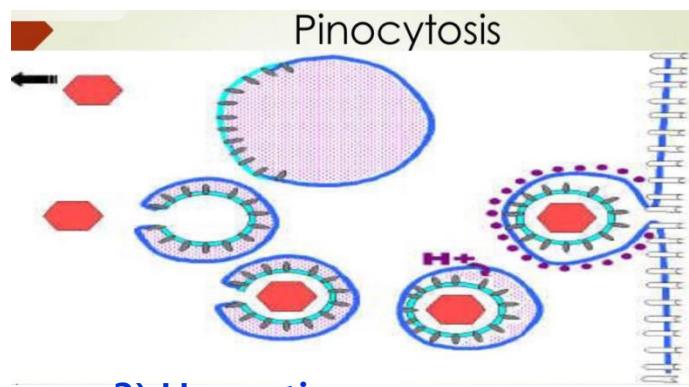


1) Adsorption:

- By **viral specialized attachment sites** distributed over the surface of the virion e.g. orthomyxoviruses and paramyxoviruses glycoprotein spikes
- adenoviruses attach through the penton fibers.
- Adsorption occurs to **specific cellular receptors** (glycoproteins, phospholipids or glycolipids)

2) Penetration:

- Pinocytosis (viropexis). obligatory for unenveloped viruses such as polio. Some enveloped viruses, e.g. **orthomyxoviruses**.
- Fusion. e.g. enveloped viruses
- Direct penetration. e.g. bacteriophage.



3) Uncoating:

- Events after penetration which allow the virus to express its genome.

4) Eclipse phase:

- Time between uncoating and the time when mature infectious viruses could be detected again.
- During it the virus direct the cell towards virus replication.

5) Synthesis of new viral components (nucleic acid and proteins):

Transcription :

The main part of the virus replication is the transcription of specific mRNA from viral nucleic acid

Translation :

Once mRNA is formed , viruses use host cell components (Ribosomes and tRNA) for translation of this mRNA into different viral components.



6) Assembly (morphogenesis):

- The new virus genomes and its structural proteins are assembled to form new virus particles
- in the **cytoplasm** as polioviruses .
- in the **nucleus** as herpes viruses .
- at the **plasma membrane** which invests the new particle to form the virus envelope (like most enveloped viruses)

7) Release of New Viral Particles:

- Cell Lysis and release of viruses e.g. polioviruses and other small RNA viruses
- Budding e.g. myxoviruses, leukoviruses and togaviruses.
- Direct passage from a cell to contiguous cells via connecting pores e.g. vaccinia viruses .

❖INTERFERENCE WITH VIRUSREPLICATION

When a cell is infected by a killed attenuated as well as intact virus .It becomes resistant to superinfection by another virus.

⌚This is called interference phenomenon which is short lived i.e. when the first virus disappears from the cells .These cells become susceptible again to infection

Types of interference: Depending upon the type of the virus involved they are:

- 1) **Auto interference:** Occurs when cell is infected with one of viruses at a high multiplicities and viruses interfere with their own replication
- 2) **Homologous interference:** Occurs between two related viruses (from the same group).
- 3) **Heterologous interference:** Occurs between unrelated types of viruses.

Mechanism: Interference may be attributed to :

1) **Viral attachment interference:**

The virus causing initial infection blocks or destructs or alters the receptor sites of the infected cell preventing adsorption of the second virus.

2) **Formation of defective interfering particles (DI particles):**

Defective interfering particles are incomplete viruses that contain only part of the virus genome .These defective particles interfere with the replication of complete virions and are called defective interfering particles .

3) **Interferon type interference:**

This type of interference is attributed to the interferon



Micro L7 : General virology 2

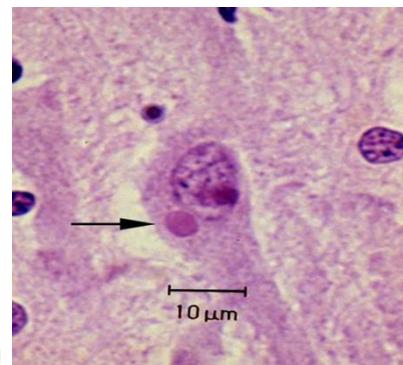
- **Inclusion bodies :** These are aggregations of viral particles in the infected cells or produced as reaction of the host cells to the injurious action of the infective virus. They are important in diagnosis of some viral diseases.
- Size : varies from 1 : 30 nm.
- Shape : may be rounded, oval or irregular in shape .
- Staining : They are stained with hematoxylin and eosin stain .
- Site of inclusion bodies :

Intra Cytoplasmic	Intra Nuclear (eosinophilic)	intra Cytoplasmic and Intranuclear
<p>* Eosinophilic :</p> <ul style="list-style-type: none"> -Negri bodies. -Gurneri bodies. <p>* Basophilic :</p> <ul style="list-style-type: none"> -Psittacosis. -Trachoma. -LGV. 	<p>* Type A (not infectious)</p> <ul style="list-style-type: none"> -Herpes zosters and simplex. -Yellow fever. -Chicken box. <p>* Type B (infectious)</p> <ul style="list-style-type: none"> -Polio. -Adenovirus. 	<ul style="list-style-type: none"> -Measles. -CMV.

* PATHOGENESIS OF VIRAL DISEASES :

- Definitions :

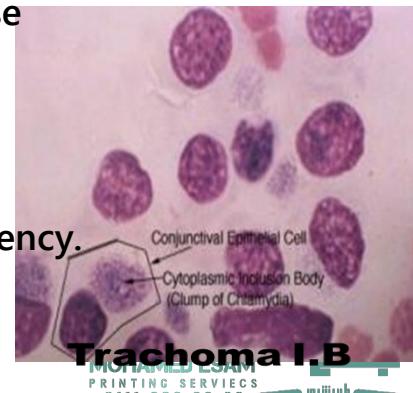
- **Pathogenicity** [Gr. "pathos", pain]: ability to cause disease.
- **Pathogen**: organism able to cause disease.
- **Pathogenesis**: means by which organism produces disease in



Negri bodies

host. A result of: injury to discrete populations of cells in particular target organs producing signs & symptoms of disease in a given host.

- **Virulence**: "capacity" to produce disease Extent of disease dependent on : Virus , dose , route of entry and replication efficiency.



Trachoma I.B



* To produce a disease, the virus should have :

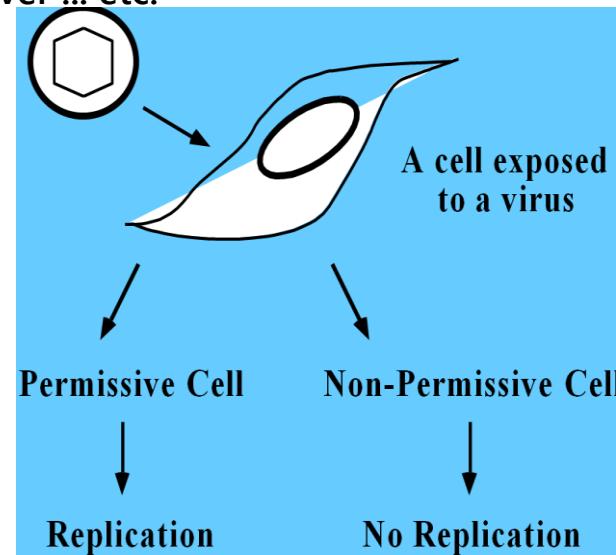
1- **Portal of entry** : e.g. The respiratory tract , skin , urogenital tract and conjunctiva .

2- **A pathway a target organ** : through the blood , lymphatics or the nerves.

3- **A target organ** : which may be CNS , skin, glands liver ... etc.

I- Dissemination of infection :

A-Local infections which occur at the portal of entry due to Primary Replication of the virus	B- systemic infection
<ul style="list-style-type: none"> - Influenza and common cold at the mucous membrane of the respiratory tract :URT . - Rotavirus infection at the GIT : diarrhea. - Papilloma virus infection of the human wartsskin . 	<p>primary replication after at the site of entry, the virus travels through the blood or lymphatics causing viraemia, or through the nerves to reach a target organ e.g poliovirus, mumps and measles.</p>



II- Pathogenicity of the viruses: When the virus comes in contact with a susceptible cell, it replicates producing cell injury which may be :

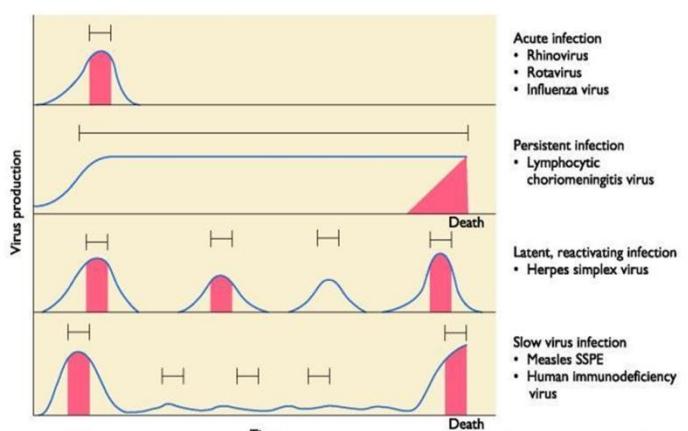
A- At cellular level .

B- At host level .

A- At cellular level :

- 1- Cell death.
- 2- Cell lysis.
- 3- Cell fusion e.g. : Paramyxoviruses & HIV.
- 4- Clusters or clumps of cells e.g.: Adenoviruses.

General patterns of infection





- 5- Inclusion body formation.
- 6-Malignant transformation.
- 7- Replication without histological changes e.g.:Rubella & Parainfluenza viruses.
- 8- Formation of viral antigens: the host immune system recognise the host cell as a foreign cell and responds against it by both humoral and cellular responses.
- 9- Latency.
- 10- Viral interference.
- 11- Ballooning of the virus e.g. HSV.

B- At host level :

- 1- Degenerative necrotic lesion e.g. poliomelitis which destroy anterior horn cells.
- 2- Proliferative changes (hyperplasia) e.g. papilloma viruses.

III- Fate of viral infections (virus host relationship):

- 1- **Apparent infection (disease)** : Here clinical signs and symptoms appear.
- 2- **Inapparent** : (abortive or subclinical) viral infection : In which there is viral infection but without overt signs or symptoms e.g. rubella , CMV acquired in utero, where this infection stopped at the viraemic stage by neutralizing antibodies.
- 3. **Persistant (chronic) viral infection** :
 - In this condition virus is continuously detected with no or mild clinical symptoms as in chronic hepatitis caused by HBV and HCV.
- 4- **Latent viral infection** : The virus persists latent or dormant but may flare up any time producing the disease as herpes viruses.

5- Slow viral infection : Here the virus have a prolonged incubation periods (months or years) , during which: virus continues to multiply with no clinical symptoms e.g.

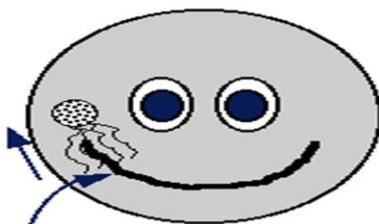
A- HIV.

B- Subacute sclerosing panencephalitis caused by a variant of measles virus



C- Non-conventional agents called prions e.g. Creutzfeldt- jakob disease and kuru (in man) and mad cow disease in cattle.

Evasion of immunity: Viral latency



Herpes simplex virus infects skin epithelial cells, then spreads to sensory neurones serving the area of infection. The immune response controls infection, but the virus persists in the trigeminal ganglion in a transcriptionally inactive, or **LATENT**, state until reactivated by sunlight, infection, hormonal changes etc.



Virus travels down axons of neurone to reinfect epithelial cells. CTL response kills infected epithelial cells..cold sore. Non-regenerating neurones express low MHC class I to prevent damage by CTL. This makes them an ideal site for latent viruses to persist.

Prions

They are clearly different from viruses hence they are :

Infectious protein particles that are composed solely of proteins with no detectable nucleic acid, E/M reveals filaments rather than virus particles.

Highly resistant to inactivation by heat, U.V. light and formaldehyde but are inactivated by hypochlorite, NaOH and autoclaving.



Normal cellular form

disease form



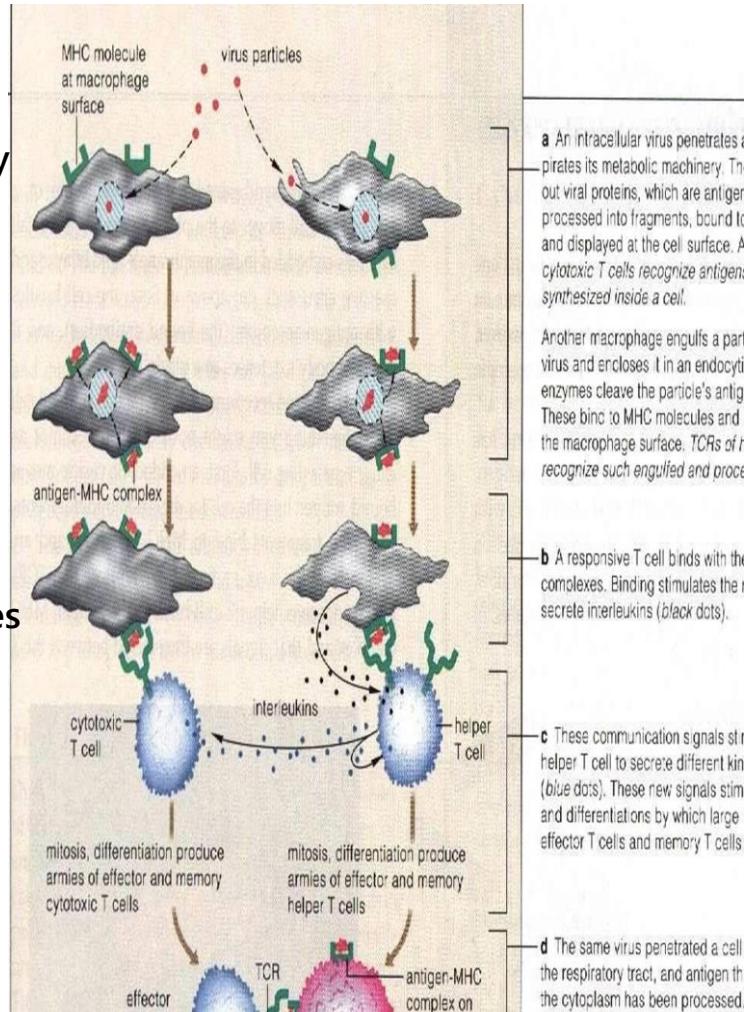
- ANTI-VIRAL IMMUNITY :

Both humoral and cell mediated immunity play a role in protection against viral infections.

Interferon is important in antiviral immunity.

I- Role of macrophages :

- Viruses gaining entry into the host via the lungs or other tissues, or traveling in the blood stream are readily taken by macrophages in the lungs, lymphnodes, liver, spleen and other blood sinuses.
- The effectiveness of macrophages in limiting viral infections depends upon their ability to prevent intracellular replication of viruses.



II- Antibody mediated (humoral) immunity:

A) Virus neutralization:

a) Viruses that have a viremic mode of spread e.g. polio, hepatitis, mumps, measles and yellow fever) are controlled by serum neutralizing antibodies (IgG and IgM) which can neutralize virus infectivity by preventing its attachment to receptor sites on susceptible cells.

- IgG and IgM neutralize viruses which circulate in the blood stream (in the stage of viraemia) before they reach the target organ as C.N.S poliovirus infections where the patient is protected from paralytic poliomyelitis.

- In the diseases of these viral infections, one attack is liable to give long lasting immunity and thus active immunization with the respective vaccine is highly effective.



b) Viruses that replicate exclusively in mucosal membranes :

- e.g. rhino and influenza viruses) in this case multiplication of the virus occurs in the respiratory mucosa despite the presence of specific antibodies in the blood.

- Secretory IgA may be important in this respect than the IgM or IgG where it neutralizes virus infectivity at the mucous surfaces. Parenteral vaccines are not effective in prevention of superficial viral infections.

- Superficial infections are not followed by long lasting immunity, and one may get several attacks during the same season due to:

- a- Weak nature of the immune mechanisms involved.
- b- The presence of several antigenic types.
- c- The emergence of antigenic variants as in influenza virus.

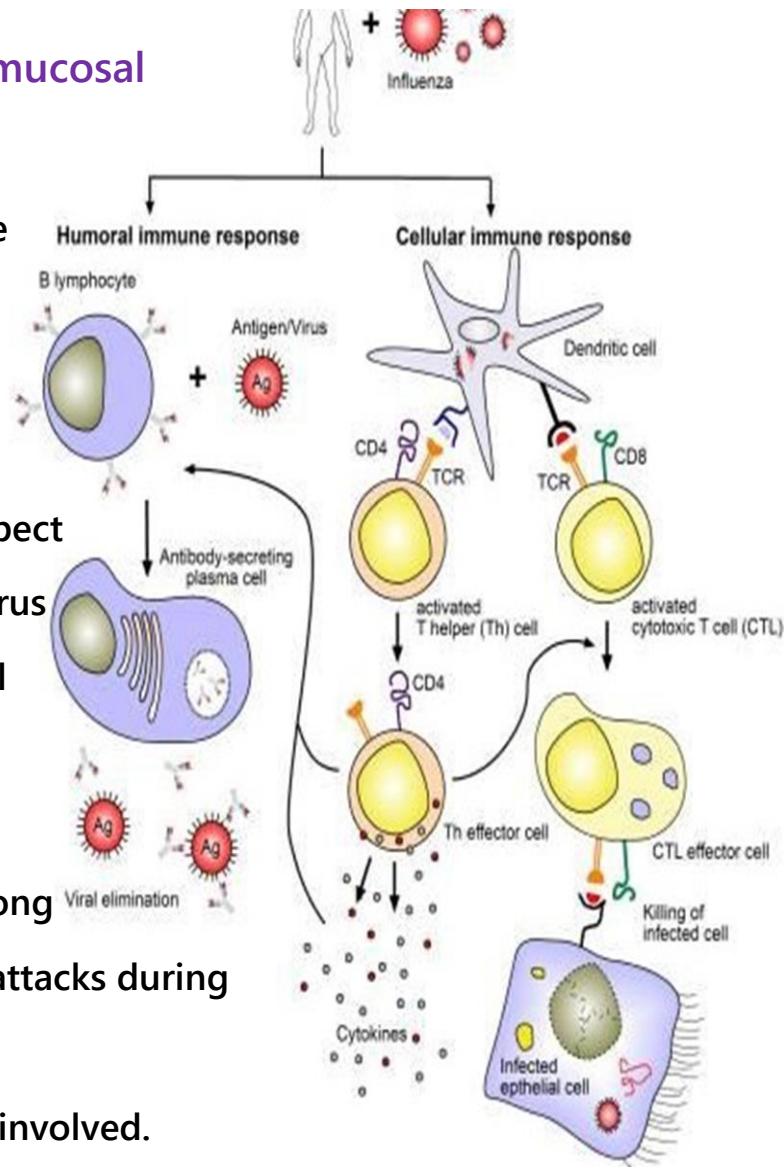
III- Cell mediated immunity (CMI):

1- Cytotoxic T cells :

Cytotoxic T cells kill virus infected cells directly after recognition of new viral antigens on the cell surface.

2- Helper T cells :

Helper T cells stimulated by viral antigens release lymphokines which have several effects of which:





- Attraction and activation of macrophages to phagocytose and destroy virus infected cells.
 - Interferon γ renders neighbouring cells immune to infection by virus.
- 3- NK cells destroy cells infected with virus. This activity is enhanced by interferon and interleukin 2.
- 4- Antibody dependent cellular cytotoxicity (ADCC) occurs when specific antibody binds to virus infected cells. Such cells are then lysed by NK cells, macrophages or polymorphs.

IV) Interferons:

Interferons (IFNs) are antiviral glycoprotein produced by the cells of all vertebrates in response to viral infection or other selected stimuli.

* Types of human interferon (Hu IFNs):

- Type I Interferon :

- IFN - α (leucocyte IFN) produced by leucocytes(B cells and macrophages).
- IFN - β (fibroblast IFN) produced by fibroblasts.

Both are produced by viral infection (RNA viruses are stronger inducer than DNA viruses), DsRNA and bacterial endotoxins.

- Type II Interferon :

IFN- γ (immune IFN) produced by T lymphocytes.

Produced in response to antigenic or mitogenic stimulation, it can be regarded as lymphokine.

- Properties of interferons:

- 1- IFNs are low molecular weight proteins with low antigenicity.
- 2- Types of IFNs are similar in size but antigenically different.
- 2- IFNs act on the cell (nontoxic to the host cell) and not act in the virus.
- 3- IFNs are found in the cell but not found in the serum.
- 4- IFNs are stable at acid pH and inactivated by trypsin.



- Mode of action :

- IFNs inhibit intracellular replication of viruses in non infected cells by inducing proteins that prevent translation of viral mRNA i.e. they block viral protein synthesis while adsorption, penetration and uncoating takes place normally.

- IFNs have no direct effect on extracellular virus.

- Activities of interferons:

I- Antiviral activities

1- They are named interferon because they interfere with the virus multiplication .

2- A virally infected cell secrete IFN to protect non infected neighboring cells.

3- Interferon is produced early after viral infection (less than 48hrs) and persists as long as the stimulus is present.

4- Interferon is not virus specific but cell specific i.e. interferon produced in cells infected with RNA virus is similar to that produced by DNA virus while interferon produced in human will protect human cells but not animal cells and vice versa.

II- Immunomodulatory action by gamma interferon : (see cytokines).

III- Antiproliferative action:

- IFNs inhibit cellproliferation andtumour growth due to their immunostimulatory effects on macrophages, NK cells etc....

- Therapeutic uses of interferon:

1- IFNs can only be used for prophylaxis of non infected cells than for cure as they can not block viral synthesis within the infected cell.

2- Treatment of viral infections e.g. Papilloma virus, chronic hepatitis due to HBV or HCV, topical application in herpetic keratoconjunctivitis.

3- Trials of combination of interferon and zidovudine in treating AIDS.



4- As a prophylactic agent against CMV infections in patients who have undergone renal transplantation and immunocompromised patients (lymphoma patients) predisposed to VZ or HSV.

5- Trials to treat cancer patients e.g. Non-Hodgkin lymphoma , cutaneous T-cell lymphoma , hairy cell leukemia.

However , IFN has toxic side effects on the gastrointestinal tract, the C.N.S., and may cause bone marrow suppression.

- Interferon in comparison with antibody:

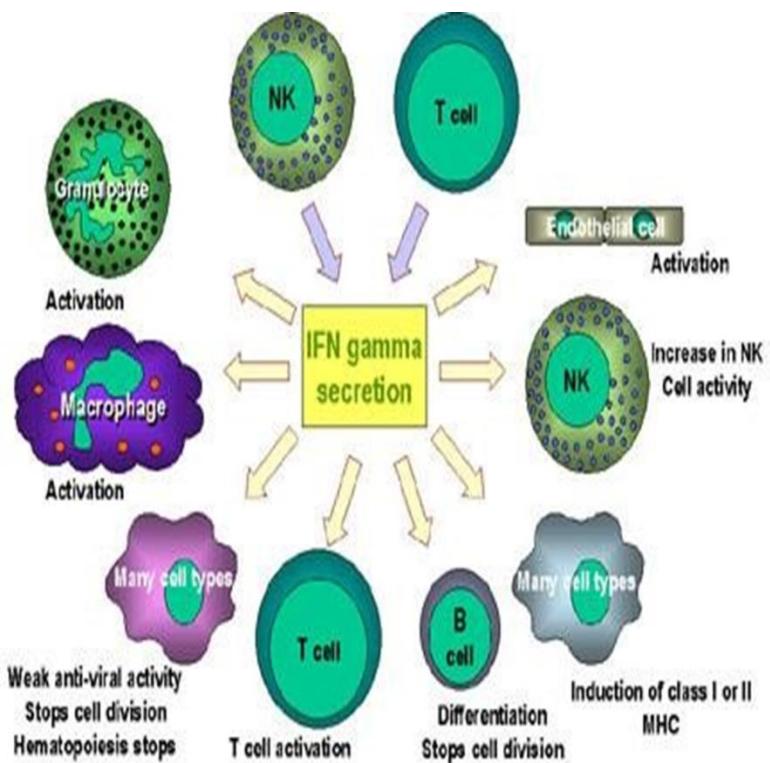
1- Interferon can be produced by any type of cell while antibody is produced by B lymphocytes (after maturation of B lymphocytes into plasma cells).

2- Interferon produced more rapidly than antibody formation.

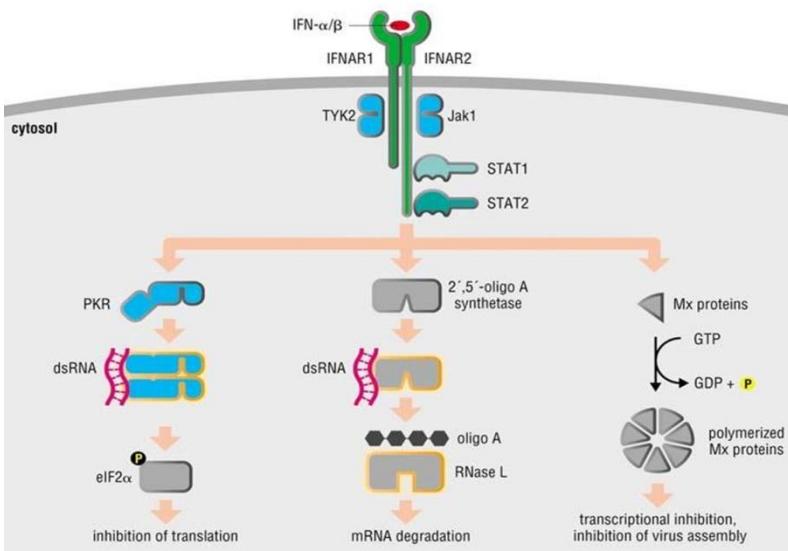
3- Interferon has no viral specificity whereas antibody is directed only to viral specific antigen.

4- Interferon can penetrate living cells and can therefore inhibit intracellular viral replication while antibody can not penetrate living cells.

5- Duration of action is short in interferon while in antibody is long.



From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson



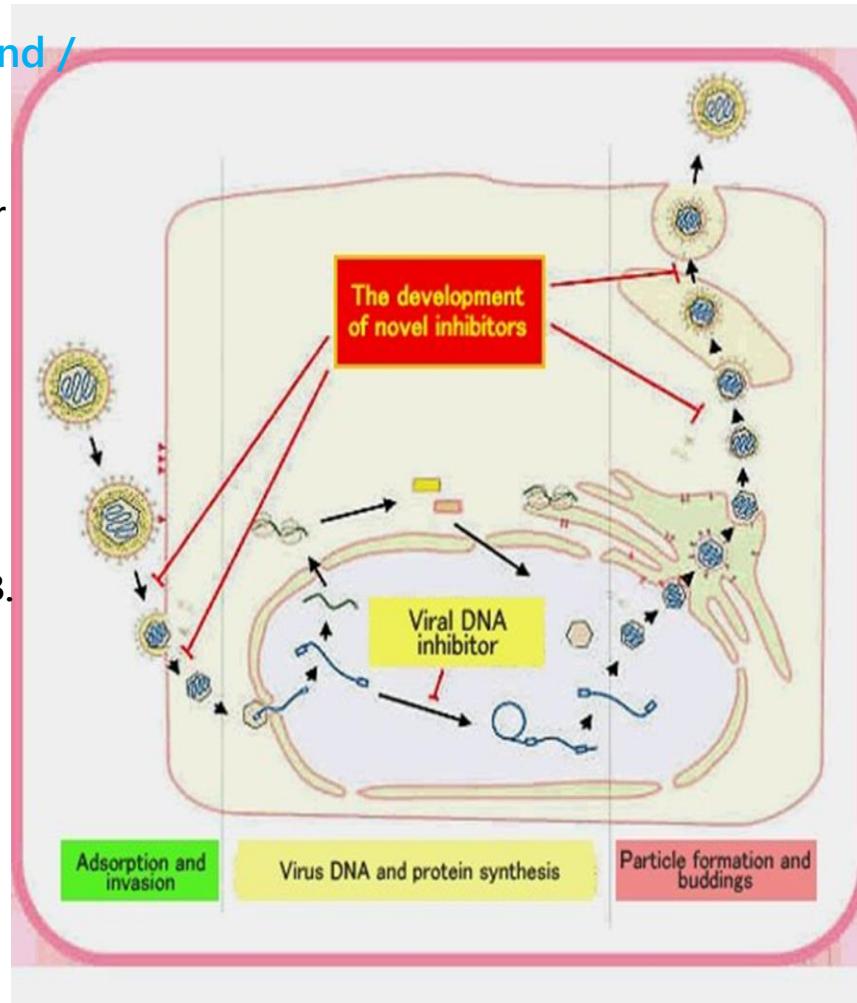


ANTIVIRAL CHEMOTHERAPY

They act by inhibiting the stages of viral replication, as follow:

I-Agents that inhibit attachment and / or Uncoating as :

- a- Anti gp120 and recombinant CD4 for inhibition of HIV.
- b- Recombinant ICAM -1 for inhibition of rhinoviruses.
- c- Neuraminidase inhibitors e.g. zanamivir for influenza viruses A and B.
- d- Disoxaril which inhibit attachment of rhinoviruses and SARS.



II- Agents that inhibit penetration and uncoating:

- Amantadine and rimantidine for prophylaxis and treatment of influenza A virus infection .

III- Agents that inhibit intracellular virus replication :

A) DNA polymerase inhibitors :

1- Nucleoside analogues:

- Idu (5- iodo-2 deoxyuridine): Used for the treatment of HSV and VZV.
- Acyclovir (acycloguanosine): It is effective when applied topically in herpes keratitis and intravenously in encephalitis or severe herpes simplex (in immunocompromised patients).



- Vidarabine: (Adenine) : Arabinose or Ara A : Similar in action and use to acyclovir.
- Ganciclovir: Similar to acyclovir but is more effective in the treatment of CMV infections.
- Famciclovir : Similar to acyclovir and can be given orally.

2- Nucleotide analogues: e.g. cidofovir which is effective against CMV and HSV.

B) Reverse transcriptase inhibitors:

1- Nucleoside analogues :

a- Zidovudine (AZT), didanosine (DDI) and stavudine (d4T) which inhibit the replication of HIV.

b- Lamivudine used for the treatment of HBV with interferon α .

2- Non nucleoside reverse transcriptase inhibitors : e.g. nevirapine.

C) mRNA synthesis inhibitors :

a) Ribavirin: for the treatment of influenza and RSV infection. Used in combination with interferon α in the treatment of HCV.

b) Foscarnet: - Effective in the treatment of CMV and HIV infection.

D) Protease inhibitors: As saquinavir, ritonavir and indinavir which inhibits HIV infection.

E) Protein synthesis inhibitors: As interferon which interferes with translation of viral mRNA into viral proteins.

CLASSIFICATION AND NOMENCLATURE OF THE VIRUSES

The most acceptable viral classifications are based on:

1)- Type of the nucleic acid (DNA or RNA viruses).

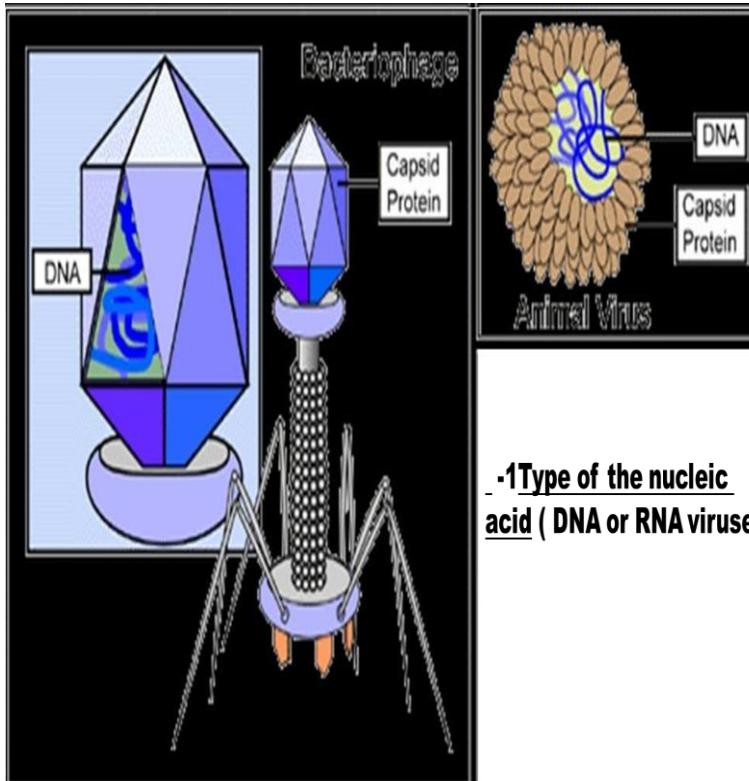
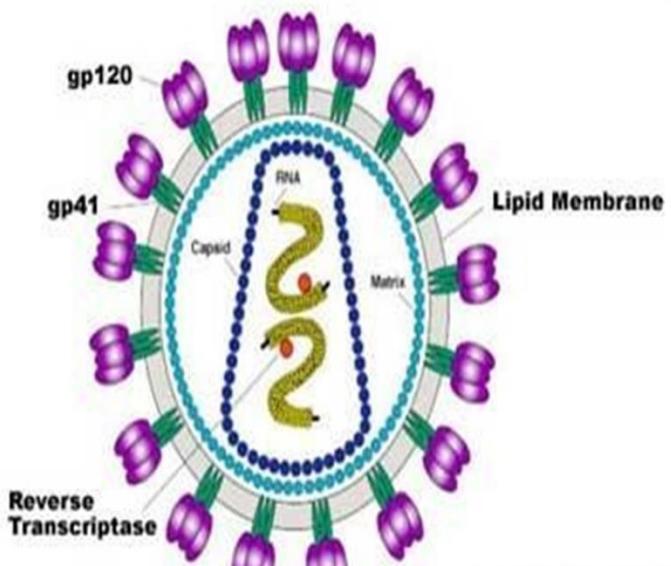
2)-Viral size : (large, medium and small viruses).

3)-Viral symmetry: (helical, icosahedral and complex symmetry).

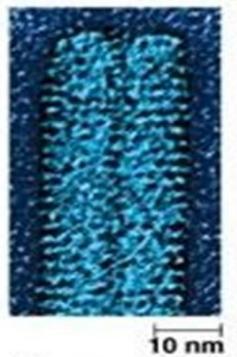
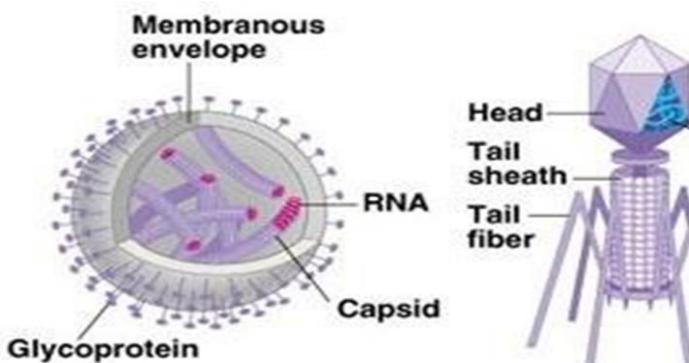
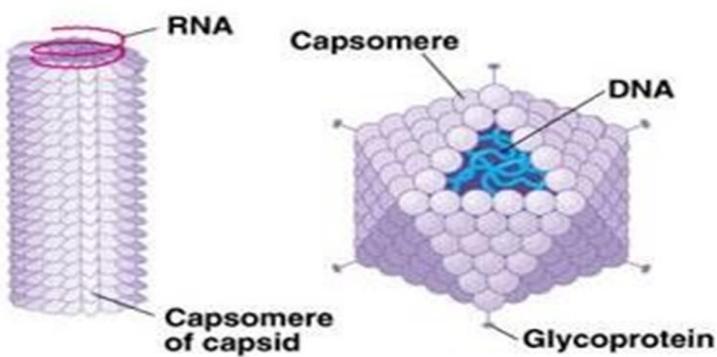
4)-Presence or absence of envelope: (enveloped or non-enveloped).



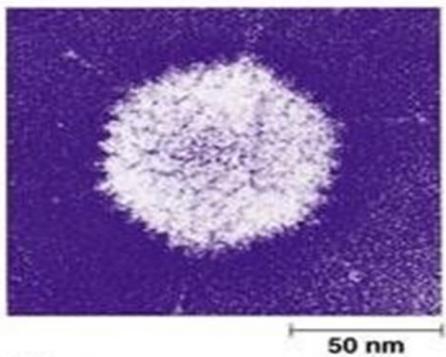
Organization of the HIV-1 Virion



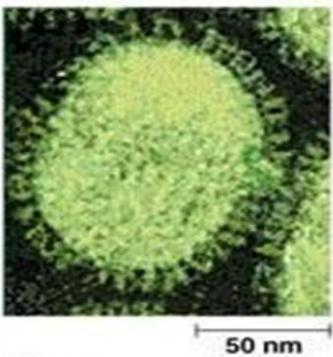
-Type of the nucleic acid (DNA or RNA viruses.)



(a) Tobacco mosaic virus



(b) Adenoviruses



(c) Influenza viruses



(d) Bacteriophag

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Viruses

General Information

Viral structure : Nucleic acid

RNA Virus Genomes

+ssRNA: If the single-stranded RNA molecules combine with ribosomes of host cells and serve directly as messenger RNA (mRNA) then the viral RNA molecule is considered to be of positive polarity.

+ss RNA

Corona virus

Arterivirus

Tobacco mosaic virus

Retrovirus

ds RNA

Reovirus

-ss RNA

Hanta virus

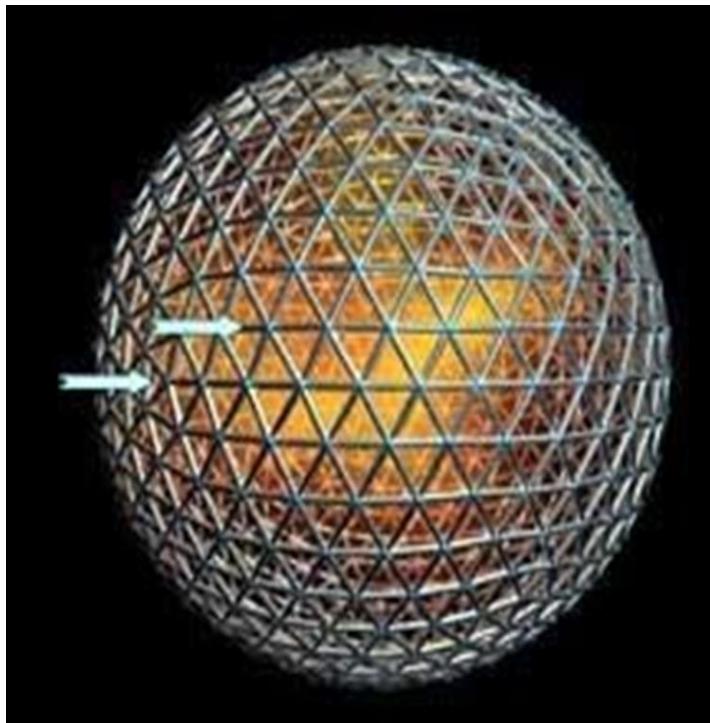
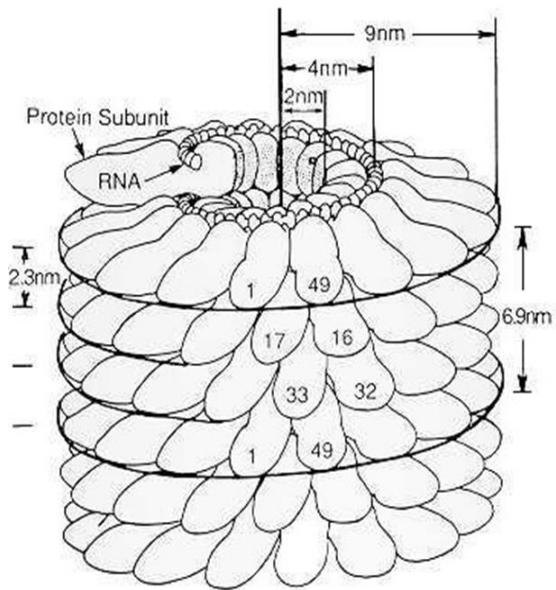
Influenza virus

Rhabdovirus

Paused

Intro Index

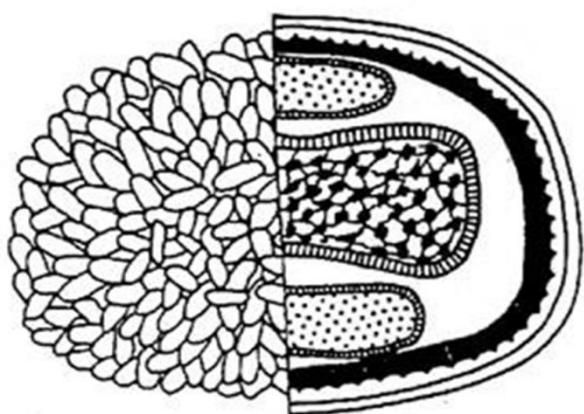
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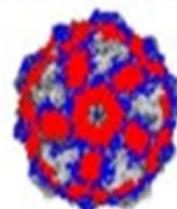
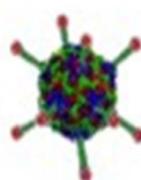
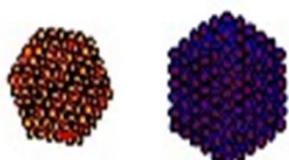
Viral symmetry: (helical, icosahedral and complex symmetry.)



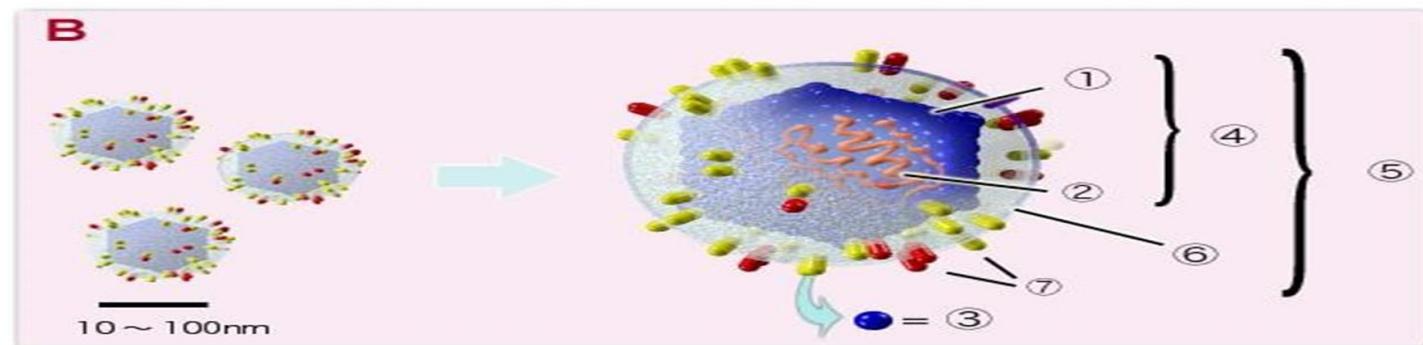
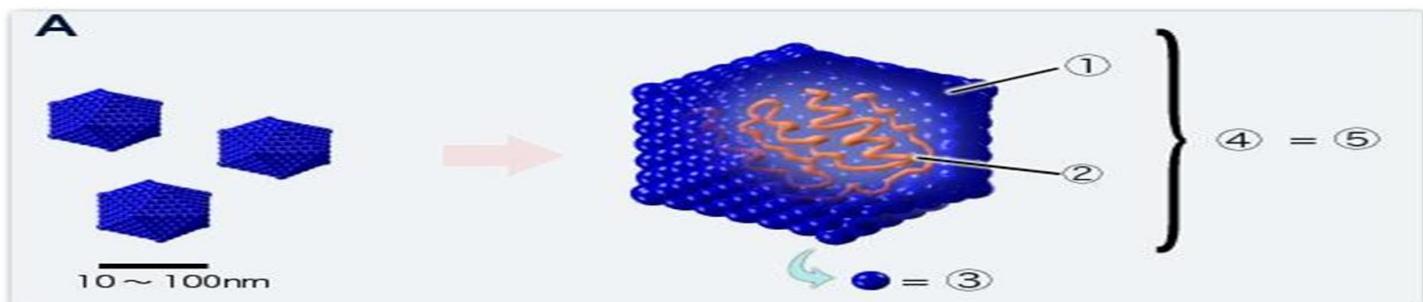
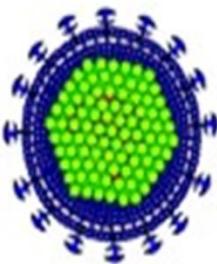
COMPLEX SYMMETRY DNA viruses RNA viruses



POXVIRUS FAMILY



Enveloped viruses



A. nonenveloped virus, B. enveloped



5)-Classification according to the disease they produce :

a) Generalized diseases :

e.g. measles, rubella, chickenpox, yellow fever ... etc.

b) Diseases primarily affecting specific organs : - Diseases of the CNS as poliomyelitis, rabiesetc.

- Diseases of the respiratory system as influenza, RSV etc.
- Diseases of the liver as hepatitis type A,B,C,D,E and F.
- Diseases of the skin or mucous membranes as herpes simplex and zoster, warts and molluscum contagiosum.

II- Nomenclature of the viruses

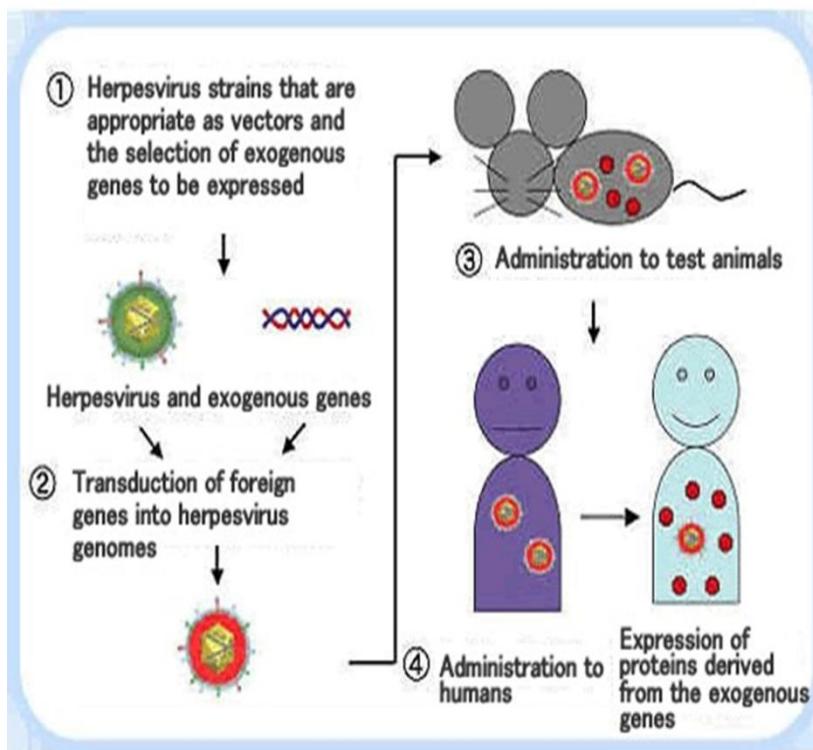
- 1) Some viruses are named according to the type of the disease they cause e.g. poxviruses and herpes viruses.
- 2) Other familial names are based on acronyms papovaviruses (papilloma – polyoma – vacuolating agents).
- 3) Others are based on morphological features of the virion e.g. coronaviruses which have a halo or corona of spikes.
- 4) Some viruses are named after the place where they were first isolated e.g. Coxackie, Marburgetc.
- 5) Some viruses are named after their discoverer e.g. Epstein – Barr virus... etc.



VIRAL VACCINES

- Types of viral vaccines:

- 1- Inactivated (Killed) vaccines.
- 2- Live attenuated virus(mutant) vaccines.
- 3- Live virulent virus vaccine.
- 4- Subunit vaccine.
- 5- Synthetic polypeptide vaccine.
- 6- Genetic engineering vaccines (recombinant vaccines).



LABORATORY DIAGNOSIS OF VIRAL DISEASES :

Two methods are used :

- Direct methods.

- Indirect method.

- Direct methods:

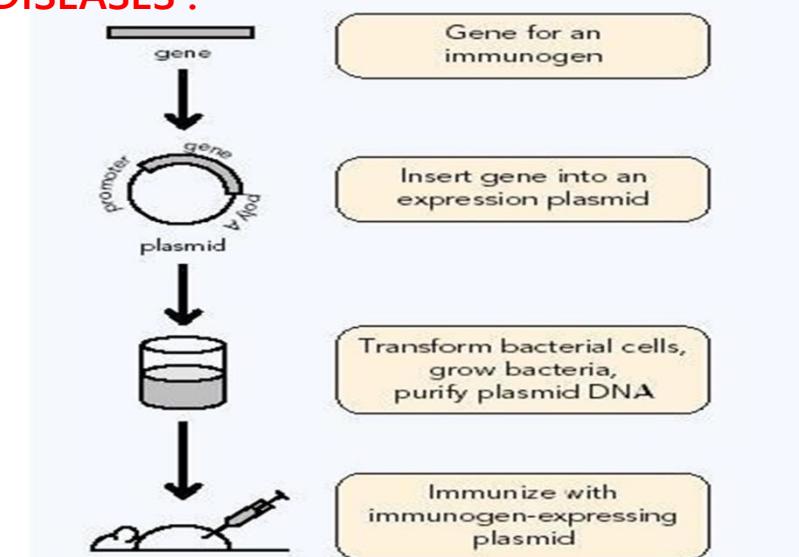
A) Virus isolation.

B) Demonstration of the virus particles.

C) Histological staining methods.

D) Detection of viral nucleic acid by

PCR or hybridization techniques.





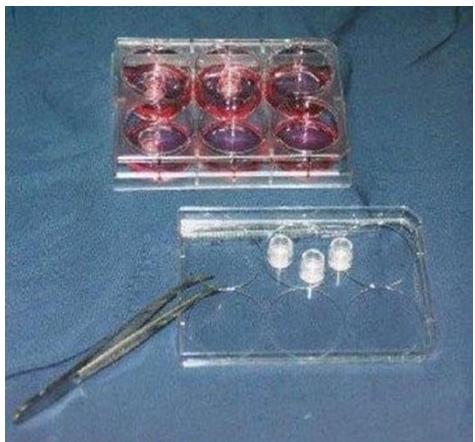
A) Virus isolation :

- Egg inoculation.
- Cell culture inoculation.
- Animal inoculation.



Laboratory animal

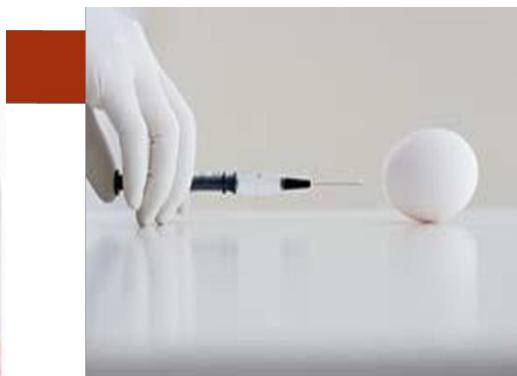
Embyronated egg



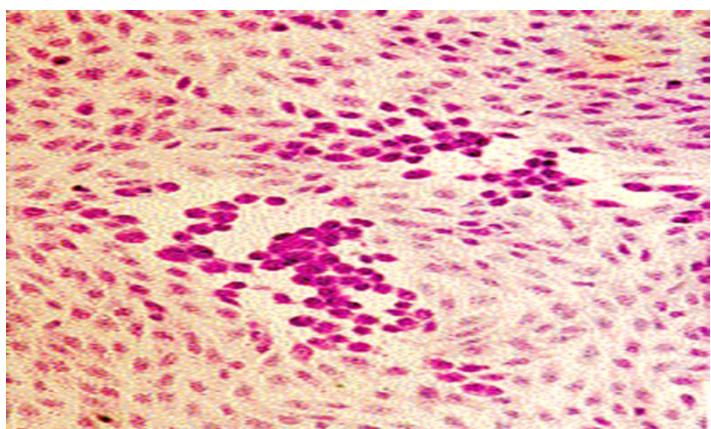
Shown: 6-well tissue culture plate with 6
single well tissue culture plate inserts



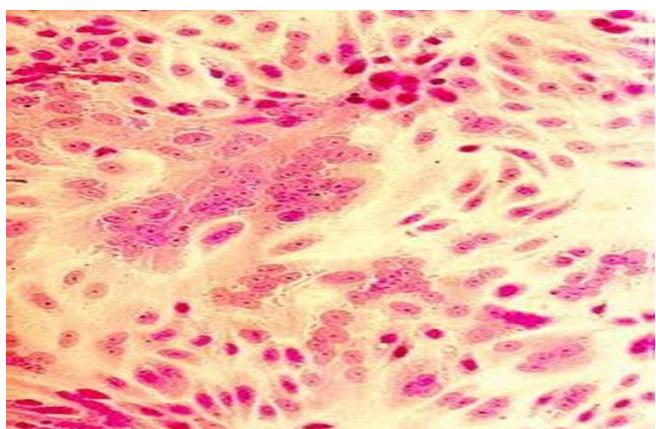
Cell culture inoculation.



Embryonated egg Inoculation.



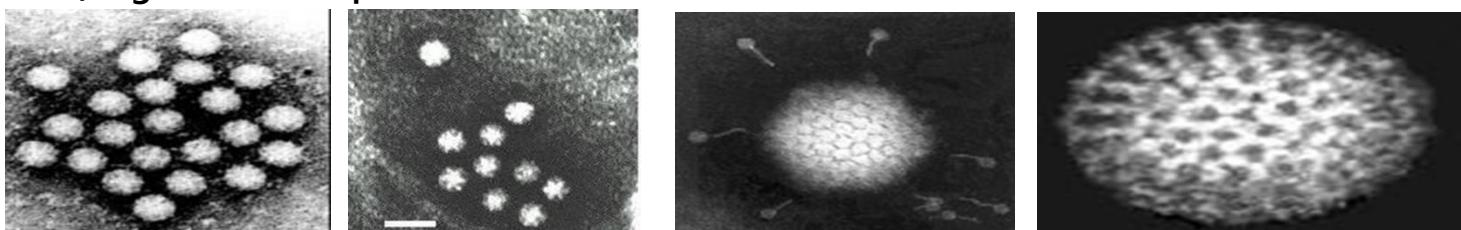
CPE of HSV showing ballooning of infected cells



Syncytia formation produced by RSV

B) Demonstration of the virus particles by:

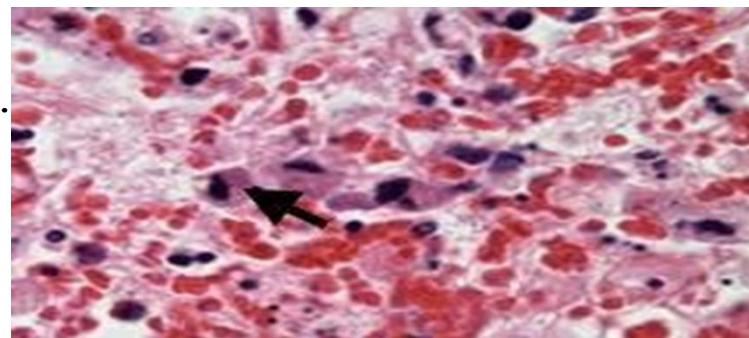
- 1- Electron microscope (EM).
- 2) Light microscope.





C) Histologic staining methods :

- a- Wright or Gimsa stain for detection of CPE .
- b- Hematoxylin and eosin for detection of inclusion bodies.



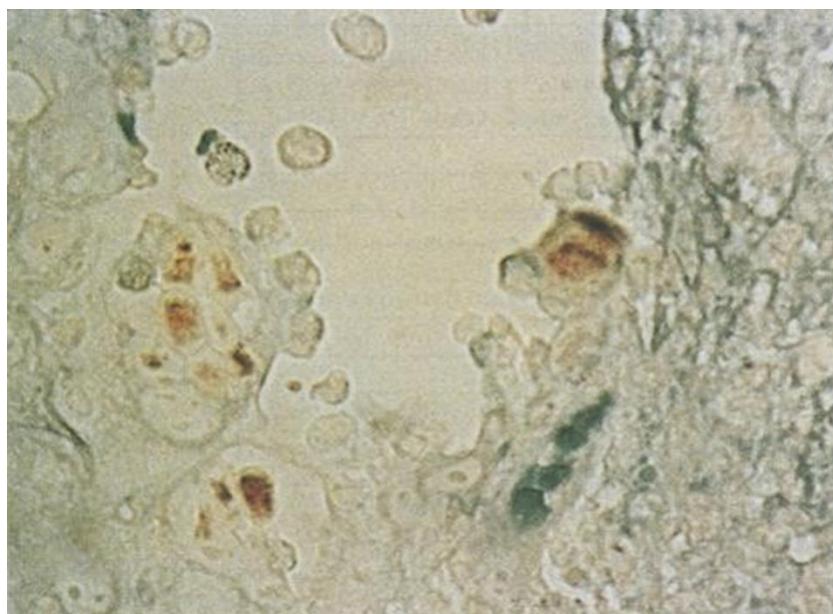
Intracytoplasmic eosinophilic inclusion bodies in rabies infected cells

D) Detection of viral genome by nucleic acid hybridization and (PCR) :

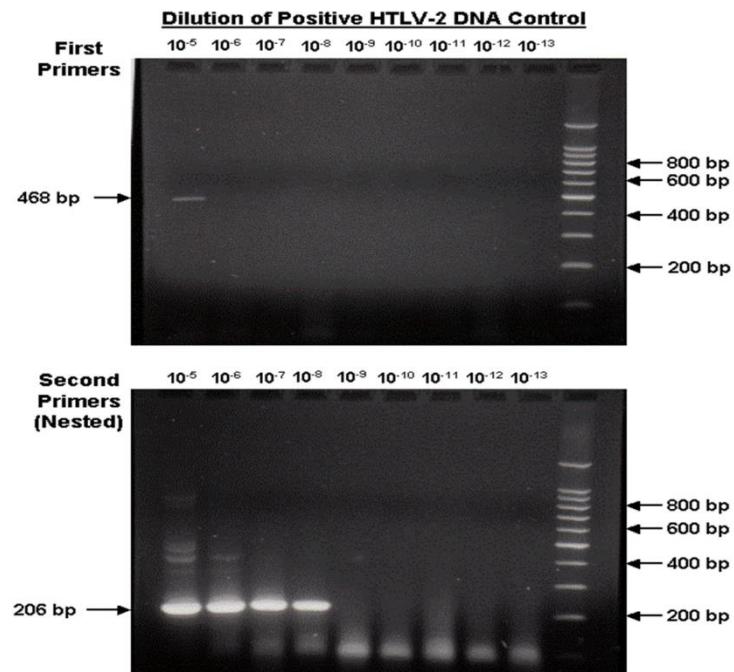
1- nucleic acid hybridization methods:

- DNA probe.
- Dot-blot hybridization.
- In situ hybridization.

2- Polymerase chain reaction (PCR)



IVP gag HTLV-2 PCR System Titration



Stained nuclei of cervical cells indicate the presence of herpesvirus DNA (in situ hybridization)



- Detection of viral antigens (viral proteins):

1) Soluble proteins.

2) Insoluble proteins.

1) Detection of soluble viral antigens :

a- Precipitin tests:

i- Gel diffusion (auchterlonyt method).

ii- counterimmunoelectrophoresis (CIEP).

b- Reverse passive haemagglutination (RPHA) tests.

c- Enzyme linked immunosorbent assay (ELISA).

d- Solid phase radioimmunoassay (SPRA).

2) Detection of insoluble viral antigens (in cells) by Fluorescent antibody tests (FAT) :

- Direct (FAT).

- Indirect (FAT).



Immunodetection of herpesvirus antigen in skin lesion.

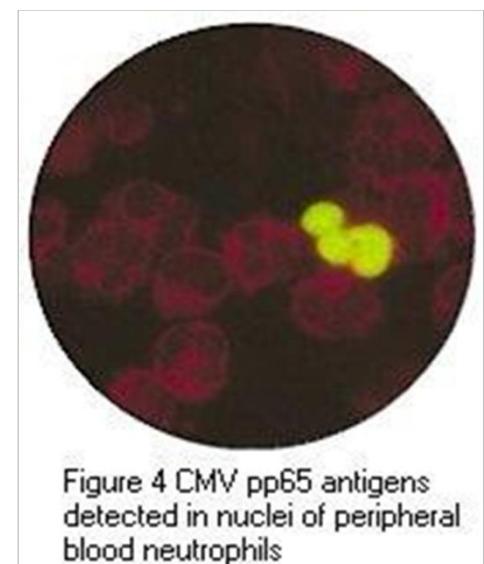


Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

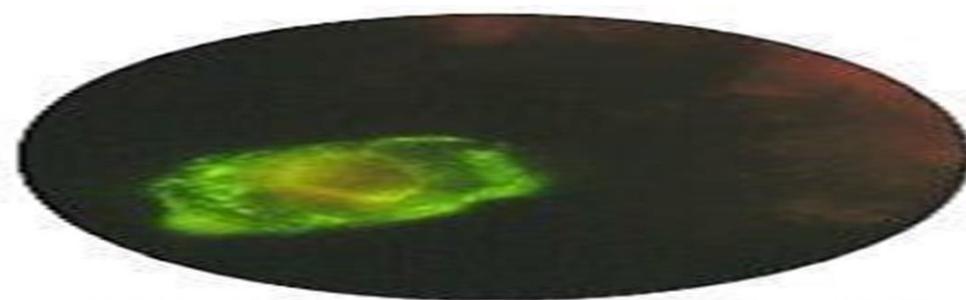


Fig. 3. HSV-infected epithelial cell from skin lesion (DFA)

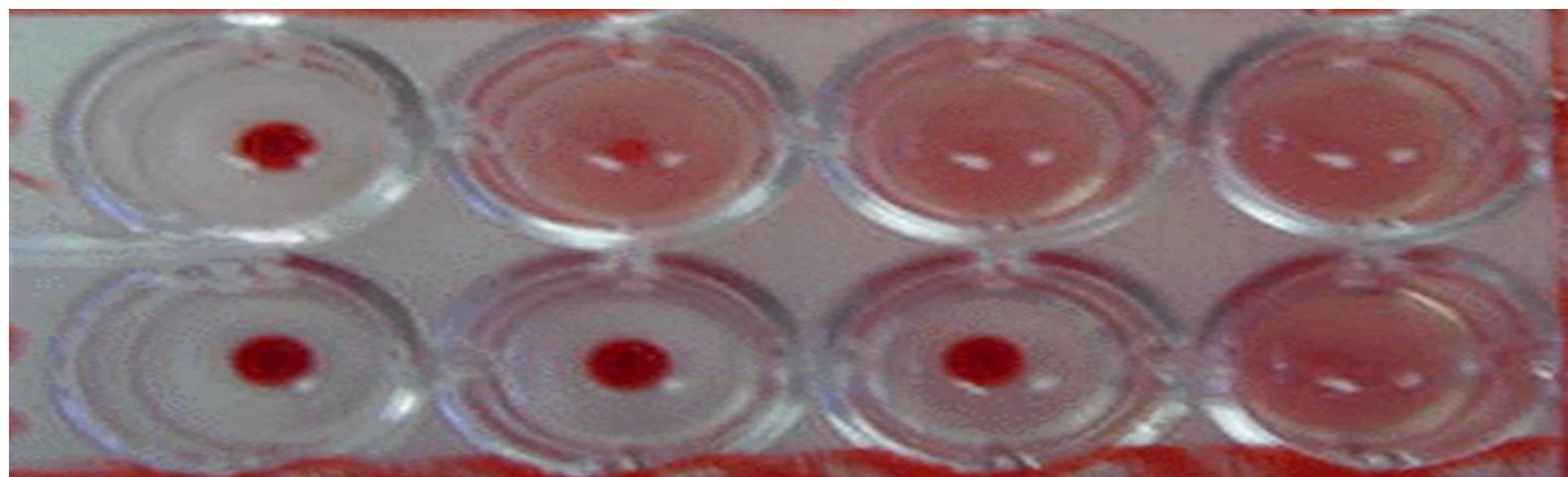
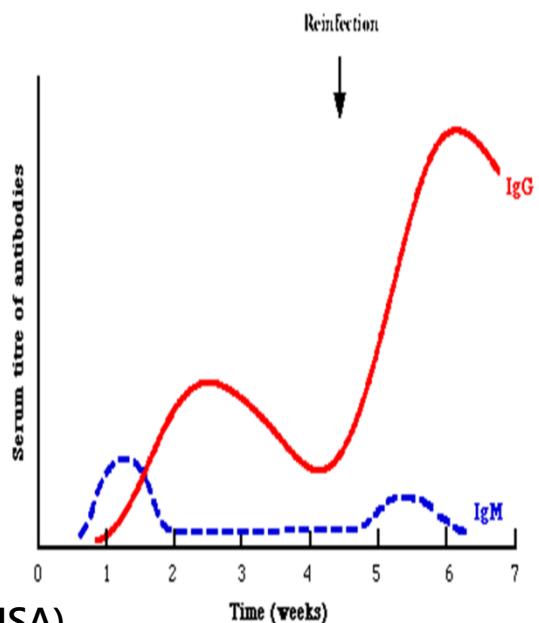


II) Indirect methods of diagnosis (serodiagnosis) :

- i) Detection of viral specific IgM in a single serum sample (rapid diagnosis).
- ii) Detection of a rising antibody titre to the virus in paired serum samples (at least four fold).

- The most commonly used serological tests are:

- 1) Neutralization test (N.T.).
- 2) Antibody analysis by Western blot.
- 3) Complement Fixation test (CFT).
- 4) Haemagglutination inhibition test (HAD).
- 5) Passive (indirect) Haemagglutination test (IHA).
- 6) Indirect fluorescent antibody (IFA) technique.
- 7) Indirect Enzyme Linked Immunosorbent Assay (ELISA).
- 8) Solid phase Radioimmunoassay (RIA).



Complement Fixation Test in Microtiter Plate. Rows 1 and 2 exhibit complement fixation obtained with acute and convalescent phase serum specimens, respectively. (2-fold serum dilutions were used) The observed 4-fold increase is significant and indicates infection.



Microtitration ELISA ,colored wells indicates reactivity

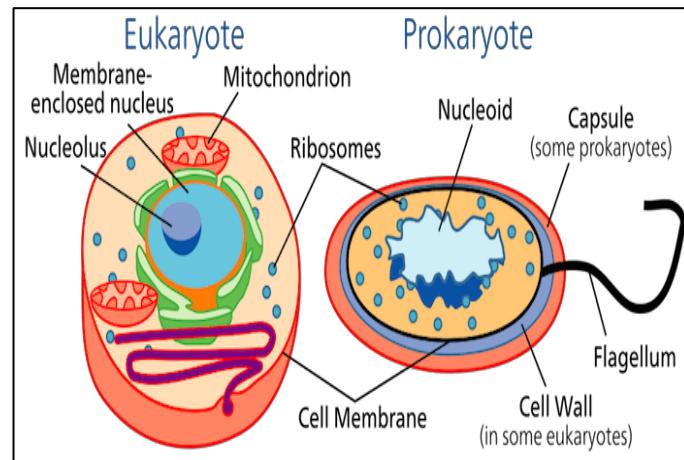




Micro L8 : General Mycology

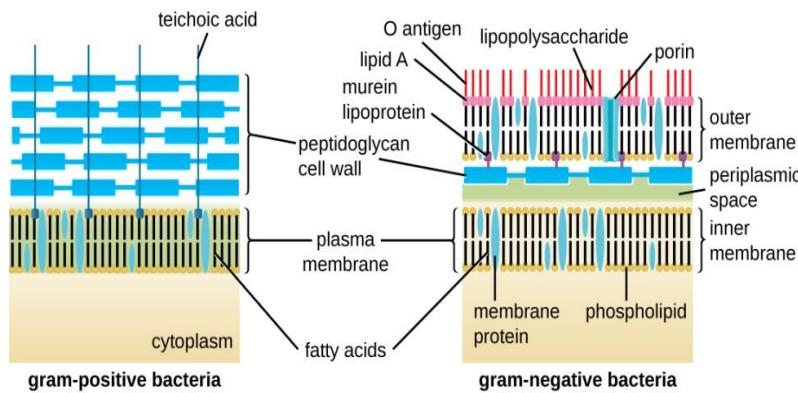
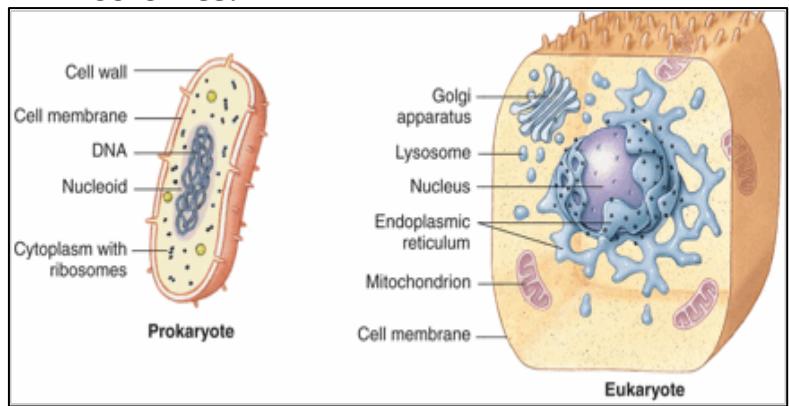
Definitions:-

- Mycology is the Study of Fungi.
- The diseases they cause are called **Mycoses**.
- Fungi can not photosynthesize their own food and so live either as:
Saprophytes or Parasites.

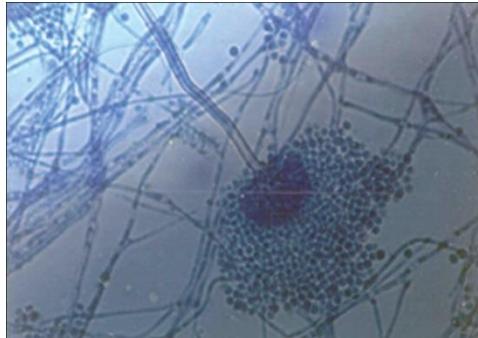
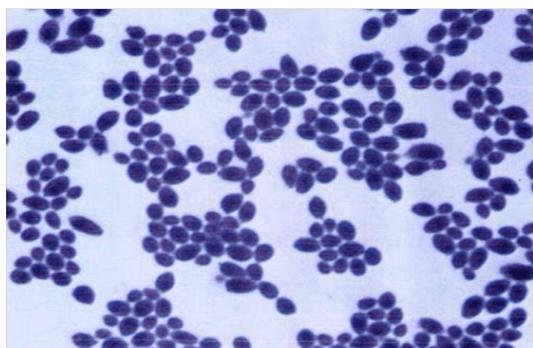
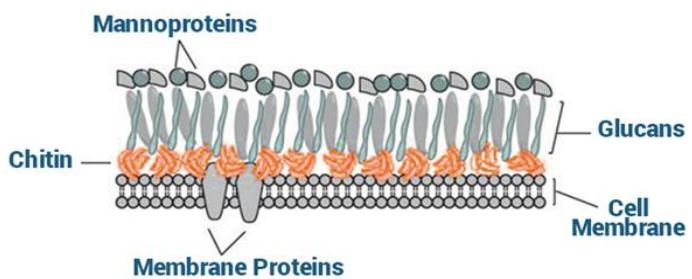


Fungi differ from bacteria in the following:

- Fungi are eukaryotic, bacteria are prokaryotic.
- Cell wall of fungi consists of a chitin-like substance while in bacteria it is formed of a muco-peptide layer.
- Some fungi (**moulds**) spread radially by the hyphae producing branching growth. Other fungi (**yeast**) produce colonies more or less similar to that of bacterial colonies.



Fungal Cell Wall





Importance of fungi :

- (1) They are common cause of damage to crops, foodstuffs, fabrics and building materials.
- (2) Few species of fungi (about 100) can cause disease in human and animals.
Fungal diseases may be due to either:
Infection, Allergies or Mycotoxins in foodstuffs.
- (3) Products of a saprophytic mould called *Penicillium notatum* and *Penicillium crysogenum* were purified and called **penicillin**.
It can inhibit growth of gram positive cocci and other organisms.
Also product of certain *penicillium* species can inhibit growth of dermatophytes (pathogenic moulds).
This product is called **griseofulvin**.



Morphological classification of fungi :

Fungi of medical importance **can be divided into three major groups**:

- **Molds** (Filamentous fungi)e.g. Dermatophytes & *Aspergillus*.
- **Yeasts** (Budding fungi)e.g. *Candida* & *Cryptococcus*.
- **Dimorphic fungi** e.g. *Histoplasma capsulatum*.



1- Mould (filamentous fungi) :

They are fungi which produce hyphae (i.e) microscopic long branching filaments.

There are 2 types:

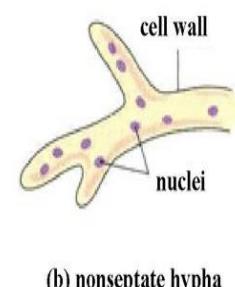
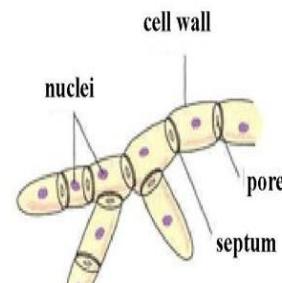
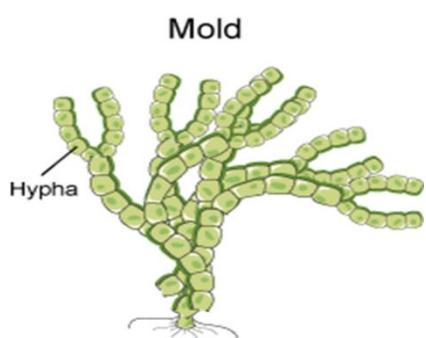
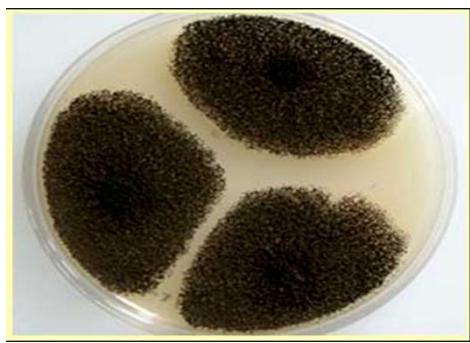
Moulds with aseptate hyphae (i.e) without cross walls in hyphae.

Moulds with septate hyphae (i.e) with cross walls in hyphae.

N.B:

- Some hyphae grow under the surface of agar and absorb nutrients they are called **vegetative** hyphae.

- The rest of hyphae grow above the surface of agar and support reproductive structures they are called **aerial** hyphae.

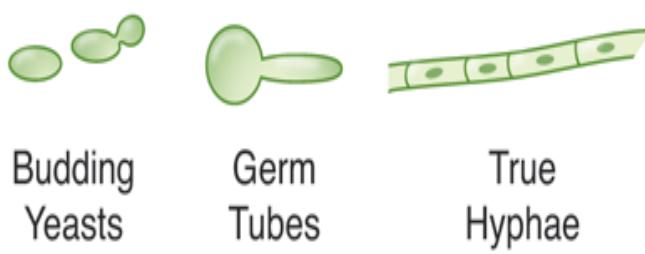
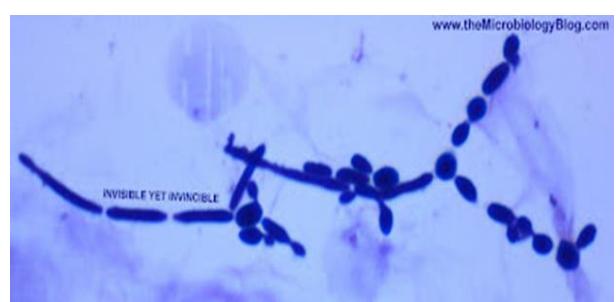
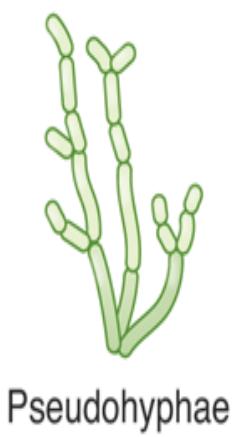
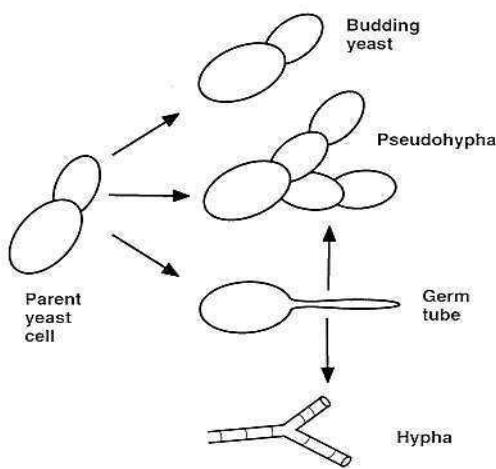


2- Yeasts (Budding fungi) :

These are fungi which reproduce by budding.

They have no hyphae but some yeasts may have elongated budding cells linked in branching chains and resemble hyphae.

They are called **pseudohyphae**.

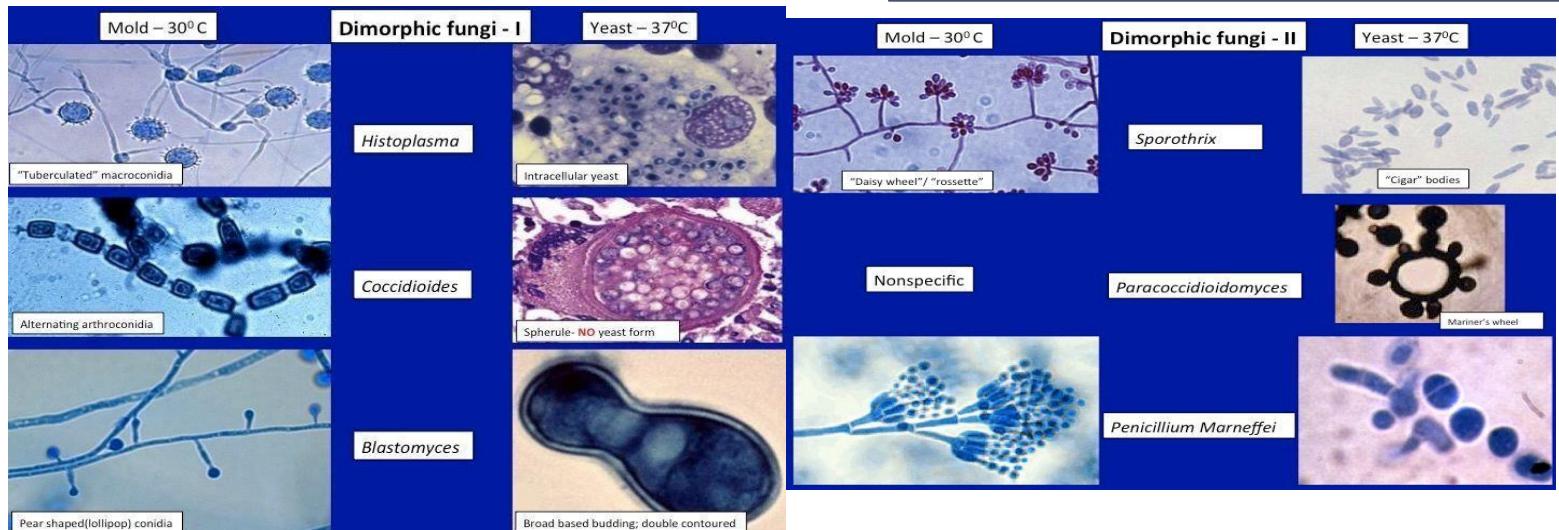
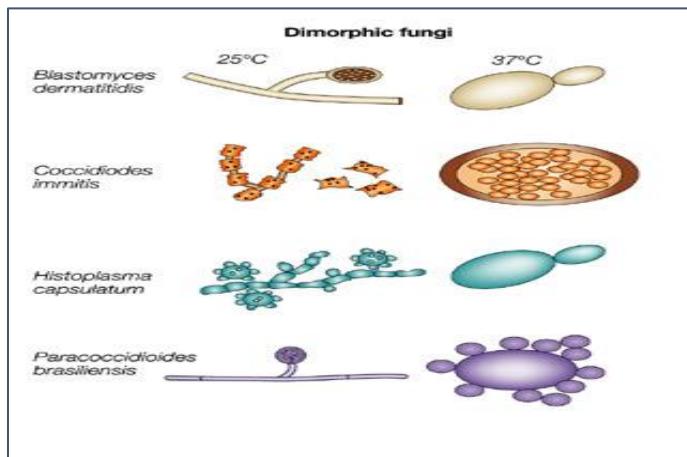




3- Dimorphic fungi :

These fungi can occur in 2 different forms:

- In nature or in culture at room temperature they occur in a filamentous form (**moulds**).
- In infected tissues or when incubated at 37°C they occur in a **yeast** form

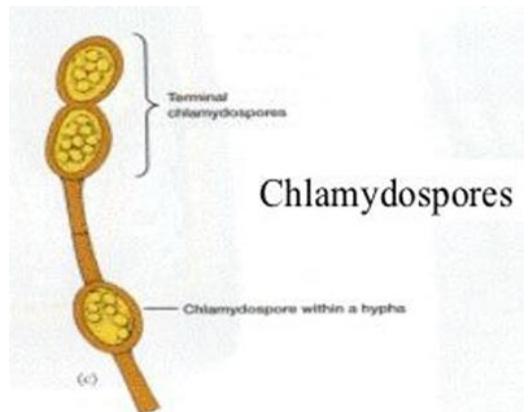


Reproduction and propagation

- Most fungi reproduce by forming **spores** (Asexual).
- The type of spore and the way in which they develop are important in the identification of the different species of fungi.

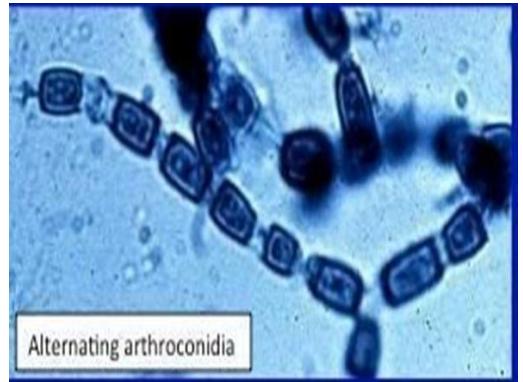
1) Chlamydospore:

A cell in the hyphae accumulates **nutrients in its cytoplasm**, become rounded and develops a thick resistant wall.



2) Arthrospore:

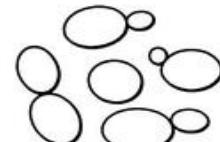
Cells in the hyphae develop thick wall and separate by fragmentation.





(3) Blastospore: (budding)

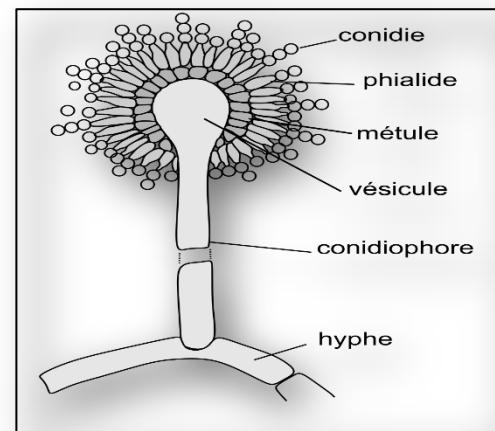
- This is the mode of reproduction in yeast.
- A small bud forms and finally separate.



Blastospores

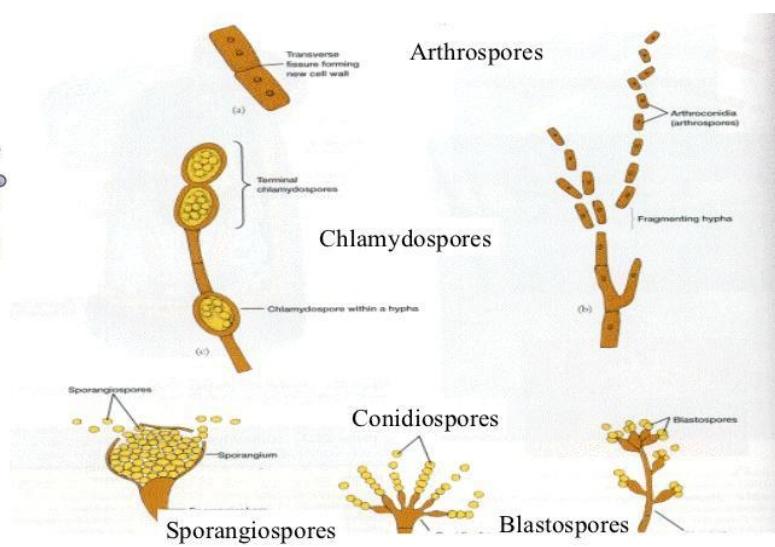
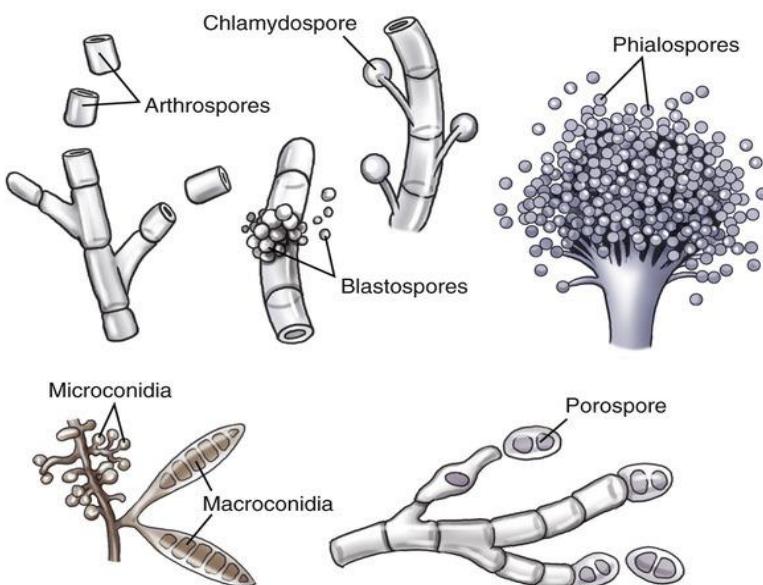
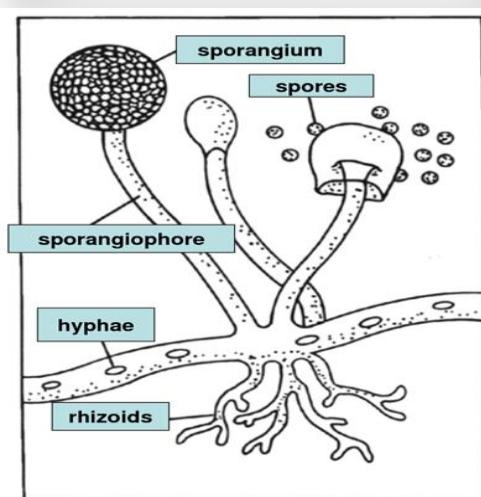
(4) Conidium:

- This is a spore produced externally on a specialized hyphae called **conidiophore**.
- The conidium becomes detached when mature.
- It may be small and called **Microconidium** or large and called **Macroconidium**.
- The shape and size of the conidia are variable and of great value in identifying individual fungi.



5) Sporangiospore:

- This is a spore produced within a swollen spherical cell (sporangium) at the end of a specialized hyphae called sporangiophore.
- Fungi which reproduce asexually are called imperfect fungi. Most of the common pathogenic species are imperfect fungi.
- Fungi which have a sexual reproductive stage are called perfect fungi.





Mycotoxins

- Mycotoxins are toxins produced by fungi.
- They are not directly related to the invasion or disease caused by fungi, they represent intoxication.
- They may cause **Acute** or **Chronic** intoxication e.g.
 - 1) Ingestion of poisonous mushrooms may cause severe or fatal damage to liver and kidney.
 - 2) Ingestion of small amounts of aflatoxin, produced by Aspergillus flavus, in contaminated foods causes chronic damage and neoplasm in liver.
 - 3) Chronic inhalation of aflatoxin causes alveolar cell carcinoma.

General criteria of mycotoxicosis:

- Not transmissible.
- No effect of antifungal in treatment.
- Seasonal.
- Associated with food ingestion.
- The degree of toxicity depends on many host factors.
- Examination of the food reveals fungal growth.



Micro L 9: Nosocomial Infection and Infection Control

Learning outcomes

By the end of the lectures, the students will be able to:

- Factors underling hospital acquired infection.
- Chain of infection
- List source of transmission of infection
- Types of Nosocomial infections
- Investigation of outbreak of hospital infection
- Needle stick injury

Hospital Acquired Infection

Nosocomial infection:

The infection acquired after admission to hospital or other health care centre.

Hospital host factors:

1. **Age:** Common in extreme age
2. immunodeficiency **diseases** (Immunocompromised patients)
3. Immunosuppressive **drugs**.
4. **Trauma** disturbs natural host defense mechanisms.

Microbial factors:

Highly pathogenic & opportunistic organisms

Organisms usually carry multiple antibiotic resistances (Any organism can cause Hospital acquired infection)

Source of infection:

Endogenous: Organisms from own Microbial factors:

- Highly pathogenic & opportunistic organisms
- Organisms usually carry multiple antibiotic resistances.

Exogenous: From other people or environment.

(Doctors, nurses, visitors, other patients, environment)

Types of nosocomial infections:

- Surgical wound infection
- Urinary tract infection
- Respiratory tract infection
- Bacteremia
- Others: Gastroenteritis- Hepatitis



Investigation of outbreak of hospital infection:

Microbiological investigation:

1. Isolation of causative agent
2. Identification of isolated organism

Epidemiological identification:

- | | |
|--------------------|--------------------------------------|
| 1-Biotyping | 2- Antibiotic susceptibility pattern |
| 3-Serotyping | 4-Bacteriocin typing |
| 5-Molecular typing | 6-Phage typing |

Infection Control

It prevents nosocomial or healthcare-associated infections.

Tools are provided to health care workers to enable them to protect themselves from the transmission of infections.

It includes:

1. Standard precautions: to all patients
2. Additional precautions: to patients infected with highly infectious pathogen.

Items Of Infection Control

- Hand washing Cleaning, Disinfection, and Sterilization.
- Personal Protective Equipments. (PPE)
- Vaccination.
- Post-exposure prophylaxis
- Dealing with emerging infections.
- Isolation

Hand washing

it has the first priority in preventing infections when providing care for a patient.

Types of Hand Washing:

- Routine hand washing with plain soap.
- Hygienic (Antiseptic) hand wash using alcohol, chlorohexidine, (betadine).
- Alcohol based hand rub using alcohol gel.
- Surgical hand wash.

Technique of hand washing:

- | | |
|----------------------------------|---------------------------|
| 1-Palm to palm. | 2-Rt palm to left dorsum. |
| 3-Lt palm to right dorsum. | |
| 4-Fingers interlace. | |
| 5-6-Back of fingers to palm. | |
| 7-8-Rotational rubbing of thumb. | |
| 9-Rotational rubbing of palms | |



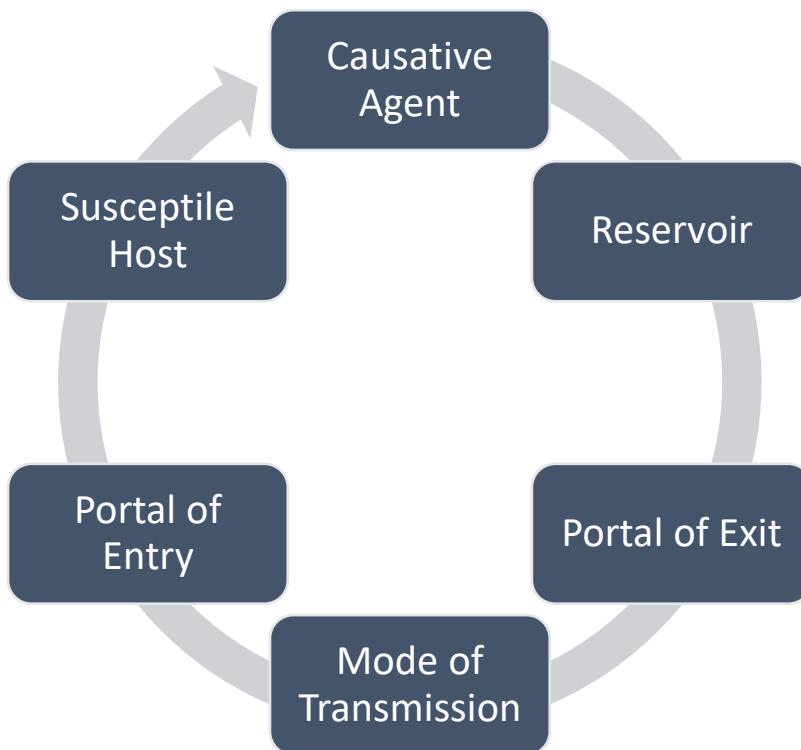
Personal Protective Equipments (PPE)

Specialized clothing or equipments worn by a worker for protection against a hazard
PPE prevents contact with potentially infectious materials by creating a physical barrier between the potentially infectious materials and the healthcare workers.

- PPE include:**

Gloves, Gowns, Bonnets, Shoe covers, Face shields, Goggles, Masks, And Respirators.

The Chain of Infection



Vaccination

Hepatitis B vaccine

A course of **three (3) vaccine** injections are given with the second injection at least one month after the first dose and the third injection given six months after the first dose. Afterward an immune system antibody to HBsAg is established in the bloodstream. The antibody is known as **anti-HBsAb**. This antibody and immune system memory then provide immunity to hepatitis B infection.

The Vaccines are produced by inserting the gene for HBV into common baker's yeast where it is grown, harvested, and purified (**Recombinant vaccine**).

HBV infection cannot occur from receiving hepatitis B vaccine.

These vaccines are given **intramuscularly**.



Recommended populations.

Many countries now routinely vaccinate infants against hepatitis B.

In countries with high rates of hepatitis B infection, vaccination of newborns has not only reduced the risk of infection but has also led to marked reduction in liver cancer. Babies born to mothers with active hepatitis B infections ([within 48 hours of birth](#)), newborns are vaccinated with hepatitis B surface antigen (HBsAg) and injected with hepatitis B immunoglobulin (HBIG).

Response to vaccination

People whose antibody level is [below 10 IU/ml](#) should be tested to exclude current or past Hepatitis B infection, and given a repeat course of 3 vaccinations, followed by further retesting 1–4 months after the second course.

Those who still do not respond to a second course of vaccination may respond to [intradermal](#) administration or to a high dose vaccine or to a double dose of a combined Hepatitis A and B vaccine.

Those who still fail to respond will require [hepatitis B immunoglobulin \(HBIG\)](#) if later exposed to the hepatitis B virus.

Safety

The hepatitis B vaccine was found to be generally safe.

There have been numerous reports that the Hepatitis B vaccine is linked to [chronic fatigue syndrome](#), a syndrome marked by severe fatigue, and muscle pains among other symptoms.

Needle stick injury

A [percutaneous](#) piercing wound typically set by a needle point, but possibly also by other sharp instruments or objects.

There is a risk to transmit diseases through the passage of the

- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- and HIV, the virus which causes AIDS.

والعمر ماضٍ إنما يبقى صنيعُك والأثر



CBL 1: A case of fungal infection

1-An 18-years old white male high school student visits the school nurses office complaining of a diffuse, painful rash extending from his mid thigh to his ankle. He indicates that one of his football teammates gave him topical hydrocortisone to initially treat a minor groin rash, A KOH scraping of the lesion reveals the organisms with Septate hyphae and arthrospores.

- What is the most likely diagnosis?
- Describe the laboratory diagnostic tests ?
- Outline the treatment plan for this case?

2-An 75-year-old man, comes to clinic for a fungal nail infection of the big toe on his left foot. He shows you his foot and you observe that the affected nail is thickened and chipped and has a yellow-white color. A KOH scraping of the lesion reveals the organisms with Septate hyphae

- What is the most likely diagnosis?
- Describe the laboratory diagnostic tests ?
- Outline the treatment plan for this case?

-Definitions:

Mycoses	fungal infection
Onychomycosis	a fungal infection of the nail
Conidia	<ul style="list-style-type: none"> -Asexual reproductive structures -may be formed on specialized hyphae, termed conidiophores -microconidia small and macroconidia are large or multicellular
Arthroconidia(arthrospores)	Conidia that result from the fragmentation of hyphal cells.

-Human Mycoses: According to site of the body affected, human mycotic infections are grouped into:

1-Superficial mycoses 2-Subcutaneous mycoses

3-Systemic (deep) mycoses

1-Superficial Mycoses:

-Affect the **skin** and / or **mucous membrane, hair or nails**.

-Dermatophytosis is one of the important superficial Mycoses.



1. Tinea capitis (head)
2. Tinea faciei (face)
3. Tinea barbae (beard)
4. Tinea corporis (body)
5. Tinea manus (hand)
6. Tinea cruris (groin)
7. Tinea pedis (foot)
8. Tinea unguium (nail)



Dermatophytosis

-Incidence: It is the **commonest** of all mycoses.

-Dermatophyte infections were described as **ring-worm** or **tinea**.

-Etiology: Caused by dermatophytes which are filamentous fungi classified into 3 genera:

- **Microsporum**: 17 species. Cause skin and hair infections (**not infect nails**).
- **Trichophyton**: 22 species. Cause skin, hair and nail infections.
- **Epidermophyton**: only one species. Cause skin and nail infections (**not infect hair**).

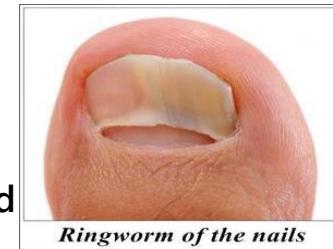


Tinea pedis
(athlete's foot)



Tinea corporis
(ringworm)

-Site of infection: Dermatophytes infect only the superficial keratinized layer of the skin, hair and nails. They never spread to deeper tissues.



Ringworm of the nails

-Source of infection: Active lesions of human - Animals - sometimes soil.

-Pathogenesis: The spores settle on the skin, germinate and form a mass of branching hyphae which grows out radially to produce circular or ring-like lesions (hence the name ringworm).



-Laboratory diagnosis of Ringworm infection:

Specimen: According to site of infection it may be; skin scrapings, nail pieces or hairs.

Direct microscopy: 1-The specimen (small pieces) placed on slide in a drop of 20% KOH

2-Cover with a glass cover. 3-Place the slide in a petri-dish with a lid together with a damp piece of filter paper to prevent dryness of the preparation.



N.B. KOH (alkali) help to **digest** the keratin surrounding the fungus so that hyphae and spores can be seen.

- In skin or nail preparations: All species of dermatophytes have a similar appearance; Septate hyphae and chains of arthroconidia (arthrospores).
- In Hair preparation: In hair, **Microsporum** species form dense masses of spores around the hair shaft (ectothrix).



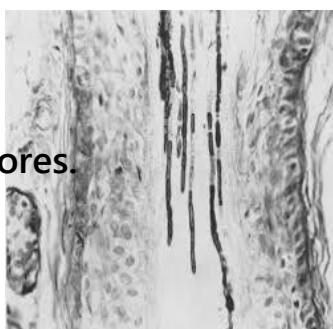
Favus:

-A severe form of tinea capitis, crusts are seen on the scalp; caused mainly by **trichophyton** sp.

-Rarely affects other parts of the body.

-It shows dead hyphae inside hair shaft with no spores.

-The dead hyphae appear as dark air spaces.





Culture: Usually done when identification of the infecting dermatophyte is required.

Culture done on: **Malt extract agar** or **Sabouraud 's dextrose agar** to which is added:

1-Actidione (cycloheximide) to inhibit saprophytic fungi. 2-Chloramphenicol to inhibit bacteria.

-Incubate at temp (22- 25C) for up to 3 weeks- Examine colonial appearance from Above & reverse.

-Iactophenol cotton blue stained film prepared & examined microscopically.

▪ Based on examination of spores (micro & macro conidia) the three genera of dermatophytes can be differentiated as follows:



1-*Microsporum*:

Both micro and macroconidia are present but macroconidia predominate.

Macroconidia are:- Spindle shaped.- With thick, rough walls.- With up to 15 septa.



2-*Trichophyton*:

-*Trichophyton* species form parallel rows of spores, arthroconidia, either outside (ectothrix) or inside the hair shaft (endothrix).

-Both micro and macroconidia are present but microconidia predominate.

-Microconidia are:- Small- Rounded or oval.- Arising from ends or sides of hyphae.

-Macroconidia are:- Club shaped.- With thin, Smooth wall.- With up to 8 septa.

3- *Epidermophyton*:

No microconidia.

Macroconidia are:- Club shaped.- With thick smooth wall.- Few septa.



Treatment

Tinea unguium: Efinaconazole solution applied topically to the nails.

Prevention: centers on keeping skin dry and cool.

Case for discussion

A 12-month-old infant presents to the outpatient pediatric clinic with erythematous scalp lesions associated with hair loss. A KOH preparation from the scraping of his lesion reveals the organisms with septate hyphae and arthrospores.

- What is most likely diagnosis?
- Describe the Laboratory diagnostic tests ?
- How to treat this case?



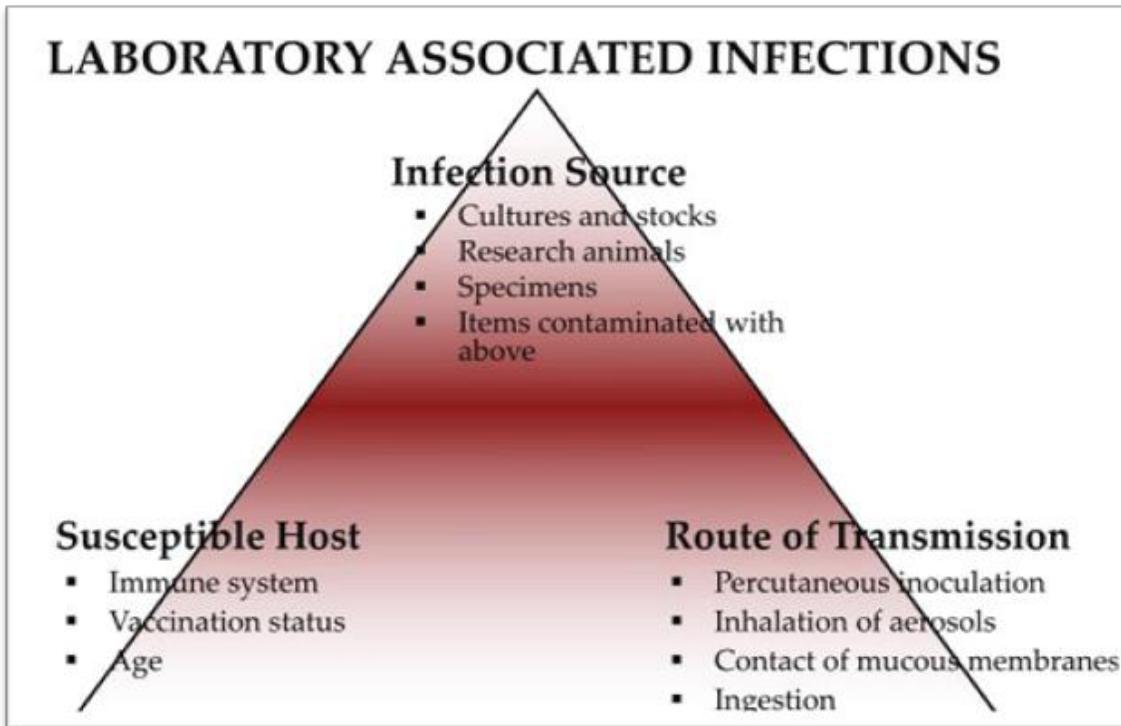
Micro TUT1: Laboratory acquired infections

Definition of Laboratory- Acquired Infections (LAI):

LAI are defined as all infections acquired through laboratory or laboratory-related activities regardless whether they are symptomatic or asymptomatic in nature.

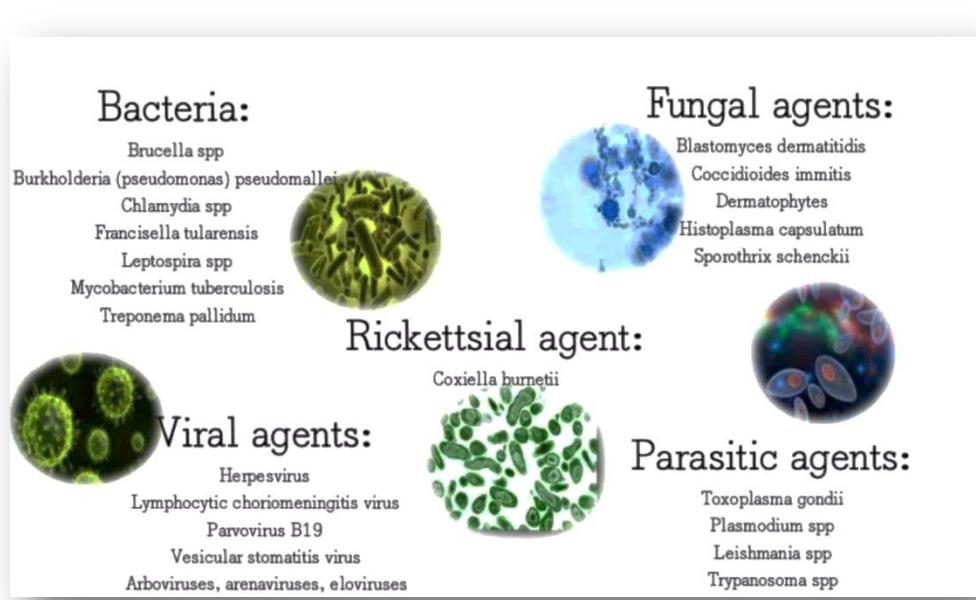
- ✓ It results from occupational exposure to infectious agents.

Risk assessment depend on:



Specific laboratory associated infections:

- Bacterial infections.
- Viral infections.
- Fungal infections.
- Parasitic infections.





Potential routes of transmission:

- Inhalation: infectious aerosols, droplets.
- Ingestion: mouth pipetting, eating, drinking.
- Percutaneous inoculation: needlesticks and other contaminated sharps; animal bites; exposure to previously broken or damaged skin.
- Mucous membrane exposure – infectious materials in contact with eyes, nose, mouth (splashes, contact from contaminated surfaces).
- Airborne: T.B. and chicken POX.
- Droplet: bacterial pneumonia and influenza.
- Ingestion: Salmonella and shigella.
- Parenteral: HIV, HBV and HCV.

Infectious Agent Exposure Protocol:

1. Exposure incident:

The following will be considered an exposure incident:

- ▶ Inhalation of aerosol and droplets.
- ▶ Failure of aerosol containment during energetic processes such as centrifugation, sonication, spilling, splashing etc. outside the biosafety cabinet.
- ▶ Mucous membrane contact.
- ▶ Contact with non-intact skin.
- ▶ Injection.

2. Immediate Response Procedures:

- Wash it off, rinse it out: Use eyewash, sink, or shower to remove the infectious material as soon as possible following an exposure incident involving contact with skin or mucous membrane surfaces.
- Notify principal investigator (PI) of the accident.
- Seek medical attention

3. Organism Data Sheet (Provided to Medical Care Personnel)

- ◎ Each infectious or toxic organism should have a prepared organism data sheet as part of this biosafety manual.
- ◎ Needle stick and sharp injuries (NSIs) are accidental skin penetrating wounds caused by sharp instruments in a medical setting.
- ◎ Sharp object: needle, scalpel, glass.



The majority of needle sticks occur when health care workers:

- Dispose of needles.
- Administer injections.
- Draw blood.
- Recap needles.
- Handle trash and dirty linens.



NEEDLE STICK POLICY:

Immediate action after injury

1) Taking care of the wound immediately after the accident:

- Let the wound bleed for a moment and then cleanse thoroughly with water or saline solution don't squeeze or suck.
- Disinfect the wound using an ample amount of soap and water followed by 70% alcohol.
- In case of contact with mucus membranes it is important to rinse immediately and thoroughly using water or saline solution only not alcohol.

2) Reporting the incident:

- ✓ It is important to report the incident immediately to the infection control team.
- ✓ This allows proper registration and subsequent management of the event.

3) Dealing with the potential source:

- ⌚ If the source of the blood is **known** the patient must be asked for permission to sample blood for **HBV, HCV and HIV test**.
- ⌚ If the patient **refused** then it must be assumed the patient is a carrier for the virus.
- ⌚ If the source of the blood is **unknown** then any blood present on the needle can be used for the serological reactions.

4) A blood sample should be taken from injured person as soon as possible after the injury.

Treatment approaches:

Management is based on finding out whether there is risk of **HBV, HCV Or HIV** depending on the serological analysis of the sample.



What to do after a potential HBV infection?

Management of the situation is based on whether or not the injured person is **immune** for HBV, either as a result of vaccination or otherwise. There are three possibilities.

Subject has full immunity, if: -

1. The person has had at least three vaccinations against HBV plus a subsequent check for antibodies.
2. The person has had hepatitis B in the past.
3. The anti HBs Ab is more than 10 IU/l.

In this case the injured person need not receive any prophylaxis.

Subject has no immunity, if: -

1. There was no vaccination against HBV at all in the past.
2. The anti HBs Ab is less than 10 IU/l.
3. 5 ml intramuscular hepatitis B immunoglobulin (HBIG) should be given within 48 hours of the injury + hepatitis B vaccine in three doses separated by one week interval

Table IV: Recommended PEP for exposure to Hepatitis B virus¹²

	HBsAg positive	HBsAg negative
Unvaccinated	HBIG*Start vaccination	Start vaccination
Vaccinated Responder HBsAb>10 IU/ml	No treatment	No treatment
Vaccinated Non-Responder HBsAb<10 IU/ml	HBIG* Start revaccination	No treatment

HBIG: Hepatitis B virus immunoglobulin

What to do after a potential HCV infection?

- There is no effective drug prophylaxis.
- The case should be followed up closely for 12 months and a serological examination for HCV should be done after 2,6 and 12 months of the incident.
- HCV is rarely transmitted from exposure of mucous membranes or non-intact skin to contaminated blood.
- Perform baseline testing for antibodies, as well as alanine aminotransferase (ALT) blood testing.
- At 4 to 6 months after exposure, retest the exposed person for antibodies and ALT.
- For early diagnosis, test viral RNA 4 to 6 weeks after exposure.
- If HCV antibodies turn positive enzyme immunoassay (ELISA) results, supplemental tests may be needed.
- If infection is identified, the exposed person must be referred to a specialist for treatment.



What to do after a potential HIV infection?

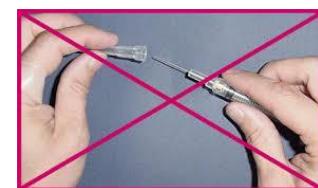
- HIV can survive in dried blood at room temperature for up to three days.
- Refer the person exposed to the risk of transmission to a trained person for medical evaluation, risk assessment and prescription of PEP.
- The decision on whether to take PEP should be based on the recommendations and counselling on adherence and on the possible adverse reactions to the antiretroviral drugs.
- Administer the antiretroviral drugs for PEP as soon as possible after the exposure (ideally within 4 hours, max 72 hours).
- Continue the PEP regimen for 28 days.
- Test for HIV antibodies at baseline, 6 weeks, 3 months and 6 months after exposure.
- If seroconversion occurs, refer the exposed person for treatment, care and support.

Highest risk:

1. Deep injury.
2. Visible blood on the device which caused the injury.
3. Injury with a needle which had been placed in a source patient's artery or vein.
4. A source patient is not on therapy and has a high viral load.

How can I protect myself from needle stick injuries?

- Avoid recapping needles.
- Promptly dispose of used needles in appropriate sharps disposal containers.
- Report all needle stick and sharps-related injuries promptly to ensure that you receive appropriate follow up care.
- Tell your employer about any needle stick hazards you observe.
- Participate in training related to infection prevention.
- Get a hepatitis B vaccination.

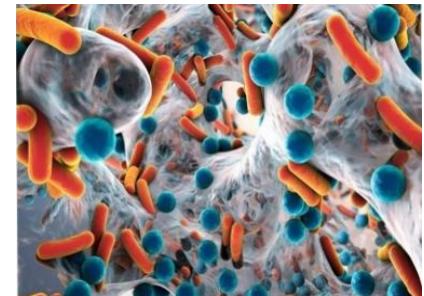




T1:Laboratory acquired infections

Introduction

- The **human microbiota** refers to the trillions of Microorganisms that reside within our bodies.
- These invisibles play a crucial role in maintaining our Health and well-being.
- This presentation will explore the diverse communities Of bacteria, viruses and fungi that make up the normal Human microbiota. Sometimes used interchangeably, these two terms have subtle differences.



The microbiome refers to the collection of genomes from all the Microorganisms in the environment.

Microbiota, on the other hand, usually refers to microorganisms that are Found within a specific environment .

What is the human microbiota?

- The human microbiota is a complex Ecosystem of micro-organisms that inhabit Our bodies, primarily in the gut, skin and Oral cavity.
- It consists of bacteria, viruses and fungi Which interact with each other and With our own cells.
- The microbiota plays a vital role in Digestion, immune system function and even Mental health .

The role of Microbiota

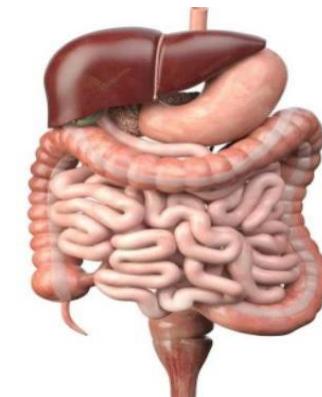
- 1-Normal microbiota provides a first line of defense Against microbial pathogens.
- 2-Assist in digestion.
- 3-Play a role in toxin degradation.
- 4-Contribute to maturation of the immune system.



5-Shifts in the normal microbiota or stimulation of Inflammation by these commensals may cause Diseases such as bacterial vaginosis, periodontitis, And inflammatory bowel disease.

The Gut Microbiota

- The gut microbiota is the largest And Most Diverse Microbial Community in the human body.
- It Is Primarily Composed Of Bacteria, with over 100 different Species.



The important functions of intestinal microbiota Can be:

- 1-Protective functions: The resident bacteria inhibit potential pathogens by Competing for nutrients and receptors or directly through the Production of antimicrobial factors, such as bacteriocins and Lactic acid.
- 2-The secretion of IgA, influence the development of the Intestinal humoral immune system
- 3-They synthesize vitamin K, biotin and folate and enhance Ion absorption.
- 4- Fermentation of food.

Quiz Time !

- What is the difference between microbiome and microbiota?
- List functions of intestinal microbiota?

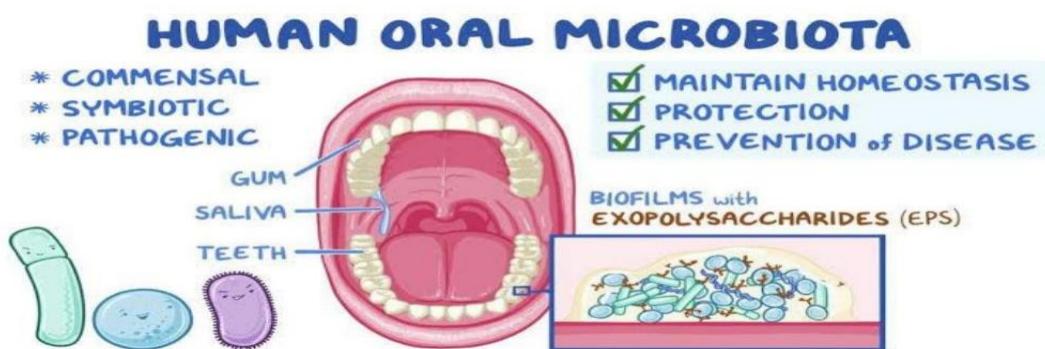
The Skin Microbiota

- The skin microbiota is a complex Ecosystem Of microorganisms that Reside on the surface of our skin.
- It acts as a protective barrier against Harmful pathogens and helps maintain The skin's PH balance.
- The Skin Microbiota Is primarily Composed of bacteria and fungi.
- Its composition can be influenced by Factors such as hygiene, environment and Genetics.



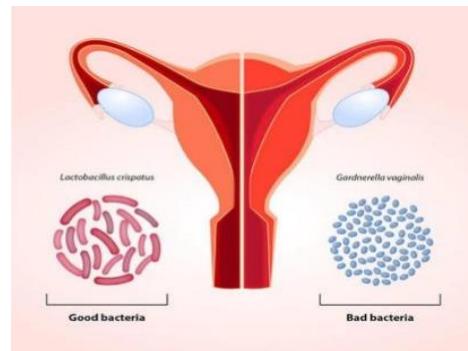
The Oral Microbiota:

- The oral microbiota is a diverse Community of microorganisms that is Present in mouth and oral cavity.
- Disturbance in oral microbiota can lead to:
 - 1-Gingivitis.
 - 2-Dental caries.



Normal microbiota of the Vagina:

They contribute to the maintenance of acid pH through the production of acid from carbohydrates, particularly Glycogen.



- Its important mechanism in preventing the Establishment of other, harmful microorganisms.
- If lactobacilli are suppressed by the administration Of antimicrobial drugs, yeasts or various bacteria Increase in numbers and cause irritation and Inflammations.



Interactions with the Immune System

- The human microbiota interacts closely With the immune system.
- It Helps Educate And Regulate The Immune response, playing a crucial role In Immune Development, Tolerance and Defense against pathogens.
- Imbalances in the microbiota can lead to Immune dysregulation and increase the Risk of autoimmune and inflammatory Diseases.

The Power of the Microbiota

- Research on the h um a n microbiota has Opened up Exciting Possibilities ForMicrobiome based therapies.
- Approaches such as, probiotics, prebiotics and Fecal Microbiota Transplantation Are Being explored to restore a n d maintain a Healthy microbiota.
- Harnessing the power of the microbiota m a y Revolutionize Healthcare And Lead To Personalized treatments.

Concept	Definition
Probiotics	Live micro-organisms that when administered in adequate amount confer a health benefit on the host.
Prebiotics	A selectively fermented ingredient that results in specific changes in the composition and / or activity of the gastrointestinal microbiota thus conferring benefit (s) upon host health.
Synbiotics	Products that contain both probiotics and prebiotics with conferred health benefits.



- **Fecal microbiota Transplantation (FMT)**

involves administration of the Whole Microbial Community From Healthy donor stool into the recipient's Intestinal tract to normalize or modify Intestinal microbiota composition and Function.

Conclusion

- The human microbiota is a fascinating and essential component of Our overall health. Its diverse communities of microorganisms have Profound effects on various aspects of our well-being, from Digestion to immune function.
- Understanding the normal human microbiota and its interactions with Our bodies is crucial for developing innovative approaches to promote Health and prevent disease.

Questions:٦٦

Complete:

- 1-Microbiota is
- 2-Common sites of microbiota
- 3-Role of microbiota in the gut
- 4-If microbiota is damaged



Micro tut3: Combating Antibiotic Resistance

LLO:

1. Identify causes of failure of antimicrobial therapy
2. Describe mechanisms and origin of drug resistance
3. Differentiate between the different types of antibiotic resistance
4. Describe the efforts of AMS to overcome the problems of Antibiotic use
5. Identify the AWaRe categorization of antimicrobials
6. Outline the role of microbiology lab in combating Antibiotic Resistance

Introduction

- The discovery of antimicrobials is one of the great advances in medicine (reduced morbidity and mortality)
- But, with widespread antibiotic use, many problems have emerged
- bacterial AMR was directly responsible for 1.27million global deaths in 2019 and contributed to 4.95million deaths
- The World Bank estimates that AMR could result in US\$1trillion additional healthcare costs by 2050, and US\$1trillion to US\$3.4trillion gross domestic product (GDP) losses per year by 2030(2)

Antimicrobial Agent:

drug, chemical, or any other substance that kill microbe or inhibit/slow the growth of microbe.

A. In-vivo:

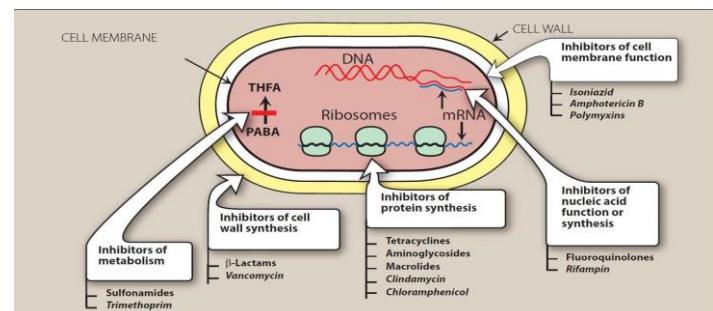
Selective toxicity: selective inhibition of the growth of the microorganism without damage to the host. it is achieved by the differences between the metabolism and structure of the microorganism and that of human cells

1. Antibacterial (Antibiotics).
2. Antifungal.
3. Antiviral.
4. Antiprotozoals, Anthelmintics

B. In-vitro:

1. Antiseptics.
2. Disinfectants

Remember: mechanism of action of antibiotics:



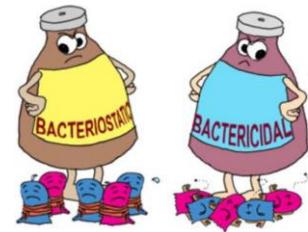


Spectrum of an antibiotic:

Narrow Spectrum Antibiotic:	Broad Spectrum Antibiotic:
Active against <i>one or very few types of bacteria.</i>	Active against <i>several types of bacteria</i>
e.g. vancomycin → gram-positive cocci (staphylococci and enterococci)	e.g., tetracyclines → many gram-negative rods, chlamydiae, mycoplasmas).

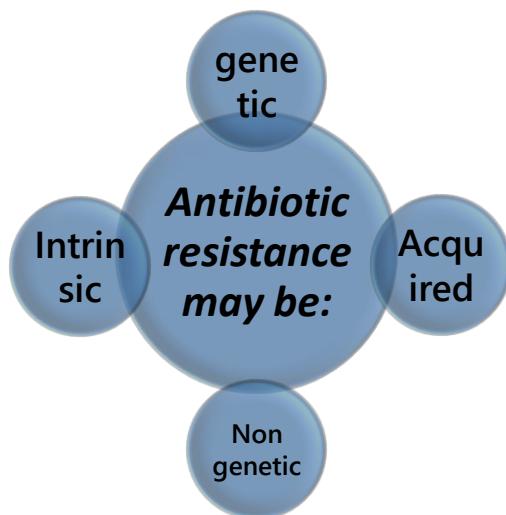
Types of Antibiotics:

bacteriostatic antibiotic:	bactericidal antibiotic:
-inhibits the bacterial growth but does not kill them. •the bacteria can grow again when the drug is withdrawn •host defense mechanisms are required to kill the bacteria.	→ kills bacteria. useful in: •life-threatening, serious infections (Endocarditis) •patients who are immunocompromised



Antibiotic Resistance:

Non responsiveness to antibiotic infection with antimicrobial-resistant pathogens makes infections harder to treat and increases the risk of disease spread, severe illness and death.





➤ NON-GENETIC RESISTANCE:

1) Bacteria can be walled off within an abscess cavity that the drug cannot penetrate effectively

2) Bacteria can be in a resting state (i.e., not dividing): e.g *M. tuberculosis*

3) The presence of foreign bodies

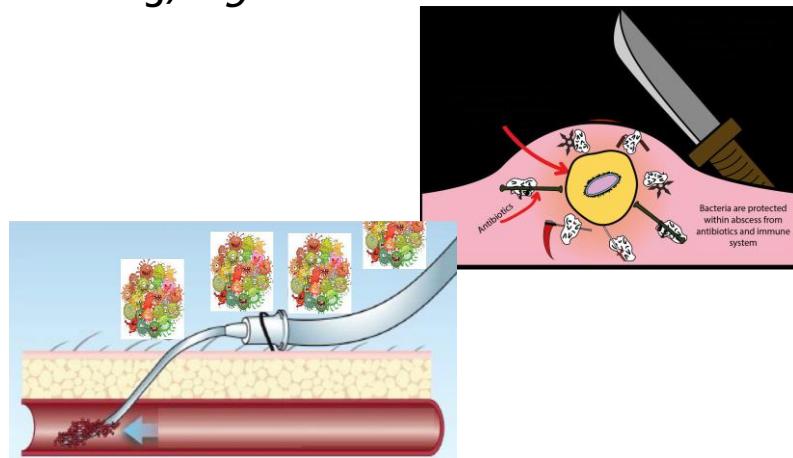
4) others:

wrong drug

the wrong dose

Noncompliance

nonadherence



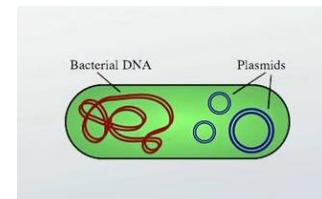
➤ Genetic resistance (bacteria express resistance genes):

1-Chromosomal mutation:

e.g. in the gene that codes for

-the target of the drug or

-the transport system in the membrane that controls the uptake of the drug.



2-Acquisition of resistance gene:

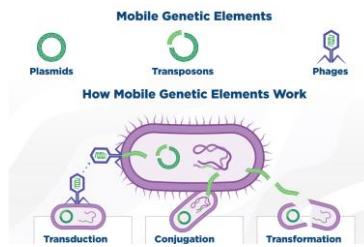
Exchange of genes between bacteria e.g. **transposon**, **phage**, **Plasmid** (resistance factors, R factors)

Plasmid-mediated resistance is very important?:

(1) It occurs in many different species, especially gram-negative rods.

(2) Plasmids frequently mediate resistance to multiple drugs.

(3) Plasmids have a high rate of transfer from one cell to another.





➤ **Intrinsic (inherent or natural) resistance:** Bacteria are naturally insensitive to an antibiotic without the acquisition of resistance factors.

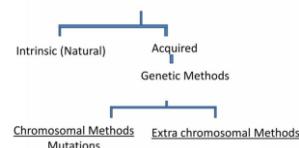
• It is consistent and can be expected once the organism is known. (*susceptibility testing is unnecessary*) examples:

1. Gram-negative bacteria resistant to vancomycin: vancomycin not penetrate the outer membrane
2. Enterococci resistant to cephalosporins: Lack of PBPs that bind to these beta-lactam agents
3. An organism lacks the target or receptor for the antibiotic (e.g. mycoplasma & cell wall active drugs)

➤ **Acquired resistance:**

- It results from altered bacterial physiology and structure due to changes in the genome of the organism.
- It is inconsistent and unpredictable → (*susceptibility testing is necessary*)
- Acquired resistance mechanisms are driven by two genetic processes in bacteria:
(1) Chromosomal Mutation (vertical evolution):
(2) Exchange of genes between bacteria (horizontal evolution)

Extent of antibiotic R. in bacteria



MDR (Multidrug resistant)

Non-Susceptibility to at least one agent in three or more antimicrobial categories

XDR (extensively drug resistant)

Non-Susceptibility to at least one agent in all but two or fewer antimicrobial categories

PDR (Pan drug resistant)

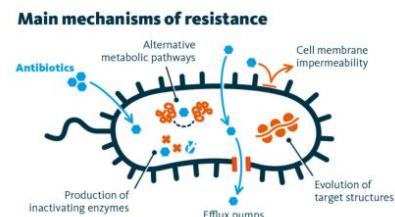
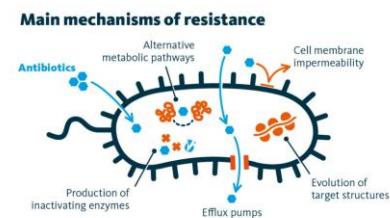
Non-Susceptibility to all agent in all antimicrobial categories



Main mechanisms of resistance

- Organism produce enzyme that destroy OR inactivate the drug:
 - ✓ Production of (beta-lactamases leads to hydrolysis of the beta-lactam drugs (penicillin & cephalosporins)
 - ✓ Production of modifying enzymes → chloramphenicol & aminoglycosides resistance.
- Organism changes their permeability to drug (Decrease influx / increase Efflux)
 - ✓ Tetracycline R.: decrease the uptake of the drug or enhance its transport out of the cell
- Organism develops an altered target for the drug
 - ✓ Alteration in penicillin binding proteins (PBPs) e.g. PBP2a in MRSA.
 - ✓ Erythromycin-resistance: altered receptor on the 50s ribosome
 - ✓ Resistance to trimethoprim is due to mutations in the chromosomal gene that encodes dihydrofolate reductase.
- Organism develops altered metabolic pathway.

Sulphonamide resistant bacteria utilize performed folic acid instead of PABA



The β -lactamases

Are enzymes produced by bacteria to destroy $\beta\beta$ lactam drugs and have different specificities

(i.e. some are more active against cephalosporins, others against penicillins)

How to overcome?

→ $\beta\beta$ -lactamase inhibitor (e.g. Clavulanic acid, sulbactam) binds strongly to β -lactamases and inactivate them. Combinations of these inhibitors and penicillins (e.g. clavulanic acid+ amoxicillin) can Overcome resistance mediated by many but not all β -lactamases.



Empiric VS directed antibiotic therapy:

➤ Empiric therapy:

- Therapy that is started before knowledge of the infecting organism's or its antimicrobial susceptibility profile
- It may be critical to the patient's health as in cases of life-threatening illnesses, closed deep seated lesion (no available sample)

The "best guess" of a causative organism is based on the following:

1. Site of infection (eg, pneumonia, urinary tract infection)
2. Age of the patient (eg, meningitis: neonatal, young child, adult),
3. Place where the infection was acquired (hospital versus community)
4. Mechanical predisposing factors (indwelling vascular catheter, urinary catheter, ventilator, exposure to vector)
5. Predisposing host factors (immunodeficiency, corticosteroids, transplant, cancer chemotherapy).

➤ Targeted antibiotic therapy:

- Chosen based on the results of culture and antibacterial sensitivity testing.
- ✓ Decrease the risk of treatment toxicity
- ✓ prevent the development of antimicrobial resistance
- ✓ reduce the cost of the treatment

Current Problems in the Use of Antibiotics:

- Inappropriate use of antibiotics
- Overuse of broad-spectrum antibiotics
- High rate of adverse effects



Global efforts to overcome Antimicrobial Resistance

e.g.

✓ **Antimicrobial stewardship**

✓ **AWaRe**

ANTIMICROBIAL STEWARDSHIP

Coordinated interventions designed to improve and measure the appropriate use of antibiotics by Promoting the selection of the optimal antibiotic, correct dose, duration and route of administration Leading to improved patient outcomes and decreased adverse events.

Basic principles of good stewardship

- Reduce inappropriate use of antibiotics
- Encourage targeted treatment with narrow spectrum drugs
- limit adverse effects

Role of AMS in Mitigating Problems and achieving the principles

Reduce inappropriate use of antibiotics

- 1.Send appropriate cultures before starting antibiotics
- 2.Empiric therapy should be tailored to the most likely pathogen(s)
- 3.Use antibiotics only when a microbiologic diagnosis indicates effectiveness

Encourage targeted treatment with narrow spectrum drugs:

- 1.Use narrow spectrum antibiotics whenever possible
- 2.Require approval for the use of advanced generation broad-spectrum antibiotics

limit adverse effects:

- 1.Stop antibiotics as soon as appropriate to reduce adverse effects
- 2.Be aware of the effect of the patient's renal function on the dose of antibiotic prescribed

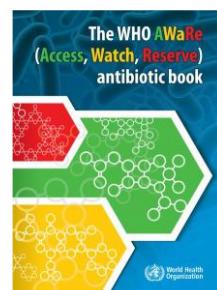


3. Be aware of the patient's hypersensitivity to specific antibiotics
4. Determine whether the patient's declared hypersensitivity is correct and clinically significant
5. Alert patients to specific unusual drug reactions, like photosensitization.

AWaRe classification of antibiotics:

Antibiotics are classified into three categories:

1. Access antibiotics that have a narrow spectrum of activity and a good safety profile in terms of side-effects.
2. Watch antibiotics that are broader-spectrum antibiotics and are recommended as first-choice options for patients with more severe clinical presentations or for infections where the causative pathogens are more likely to be resistant to Access antibiotics.
3. Reserve antibiotics that are last-choice antibiotics used to treat multidrug resistant infections.



Role of clinical microbiology lab. In combating antibiotic resistance:

- Prompt Identification of infectious agent and its susceptibility pattern
- Preparation of Antibiogram
- Alert the infection control team upon isolation of alarming resistant pathogen to limit its spread (e.g. MRSA)



Antibiogram

Summarizes the susceptibility of bacterial isolates to antibiotics during a specified period and represents the proportion of each bacterium that is susceptible to a given antibiotic.

Importance:

1. Antibiograms can help pick empiric antibiotic therapy.
2. Antibiograms help track local antibiotic resistance trends
3. Help infection control to set plans.

≥ 80% Susceptible
 60-79% Susceptible
 ≤ 59% Susceptible

Organism	Count	Hospital wide Gram Negative (GNR)																	
		Amikacin	Ampo/Clav	Ampo/Clav	Aztreonam	Cefepime	Cefotixin	Cefradine	Ceftriaxone	Cefurim	Cephobolin	Ciprofloxacin	Gentamicin	Imipenem	Mecopenem	Nitrofurantoin	Rip/Tazo	SXT	Tobramycin
Citrobacter koseri	16	100	20	20	NA	100	30	100	100	NA	20	100	100	100	100	60	60	100	
Citrobacter freundii	22	100	0	0	NA	82	8	92	92	NA	8	100	100	100	100	92	92	100	100
E.coli	772	64	50	50	NA	50	62	49	47	NA	18	73	83	96	98	82	78	49	74
Enterobacter aerogenes	28	86	9	9	NA	55	13	45	42	NA	0	86	91	98	100	18	88	96	88
Enterobacter cloacae	161	76	18	18	NA	42	4	37	37	NA	4	95	64	89	90	60	59	57	62
Klebsiella oxytoca	6	100	50	50	NA	94	62	33	30	NA	40	100	100	100	100	50	100	100	96
Klebsiella pneumoniae	478	81	76	76	NA	56	73	50	46	NA	28	86	80	93	95	57	81	58	79
Morganella morganii	10	60	1	1	NA	47	47	49	49	NA	0	80	80	60	60	0	80	60	80
Proteus mirabilis	57	83	100	100	NA	40	93	73	71	NA	62	87	80	87	88	5	87	67	78
Providencia stuartii	0	0	0	0	NA	40	0	0	0	NA	0	0	0	0	0	0	0	0	0
Serratia marcescens	65	59	17	17	NA	40	0	28	24	NA	0	86	79	90	94	6	NA	19	76