Microbiology Team
Med441

Bacterial Structure, Function and Genetics

MED441
KING SAUD UNIVERSITY

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Editing file

Color Index:
- Main text
- Boys slides only
- Girls slides only
- Doctor's notes
- Extra information
- ***Important***

Objectives

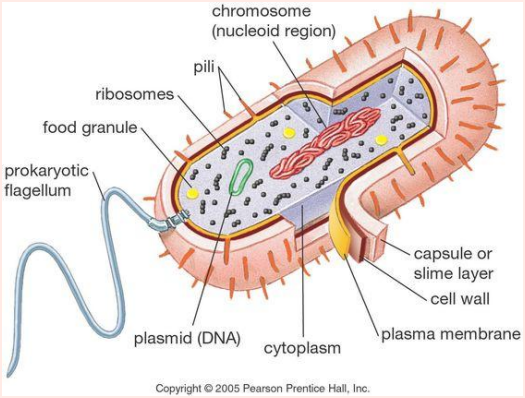
- Define the cellular organization of bacteria and recall the differences between Eukaryotes and Prokaryotes.
- Recall major structures of bacteria and its function.
- Describe the structure of cell wall of bacteria including the differences between Gram positive and Gram negative bacteria and main functions.
- Describe the external and internal structures of bacteria and their functions.
- Describe bacterial spores and its application in the practice of medicine.
- Recall basic information about bacterial genetics and replication of bacteria.

Objectives, cont.

- Describe plasmids , its origin , types and its importance in clinical practice.

- Recall genetics variations, including ; mutation and mechanisms of gene transfer and its implication on bacterial resistance to antimicrobial agents.

What is Bacteria?



- **Bacteria:** a ***heterogenous*** group of ***unicellular*** organisms.

Bacteria Properties:

Prokaryotic

Primitive nucleus

Contains plasmids (an extra piece of DNA)

Has one chromosome

About 1-8 μm in diameter

Has no mitochondria

Has no sterols

Has no nuclear membrane

Dr. Note:

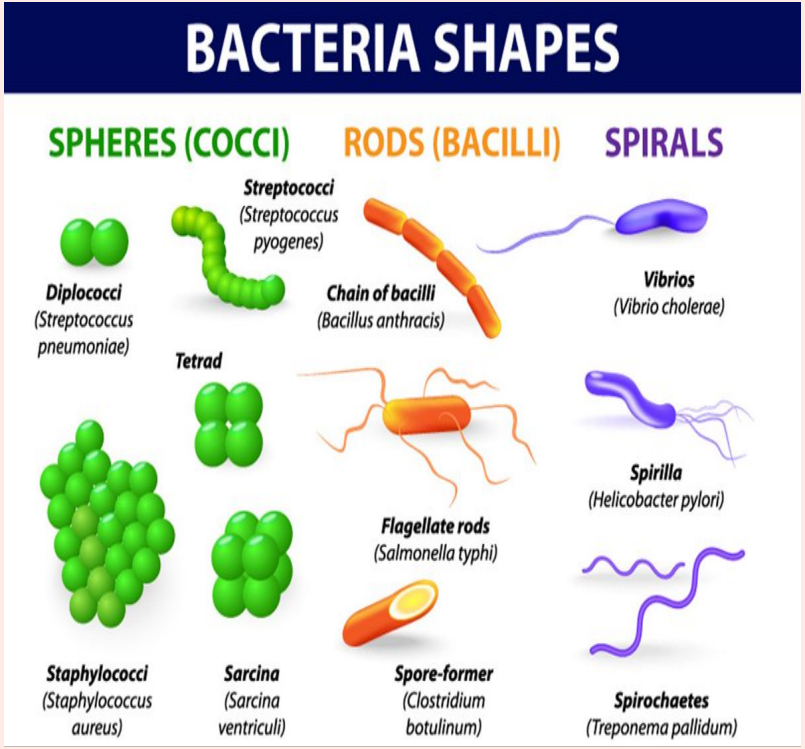
No cholesterol

Shapes & Types of Bacteria

Dr. Note:

You have to describe each shape under the microscope on scientific basis.

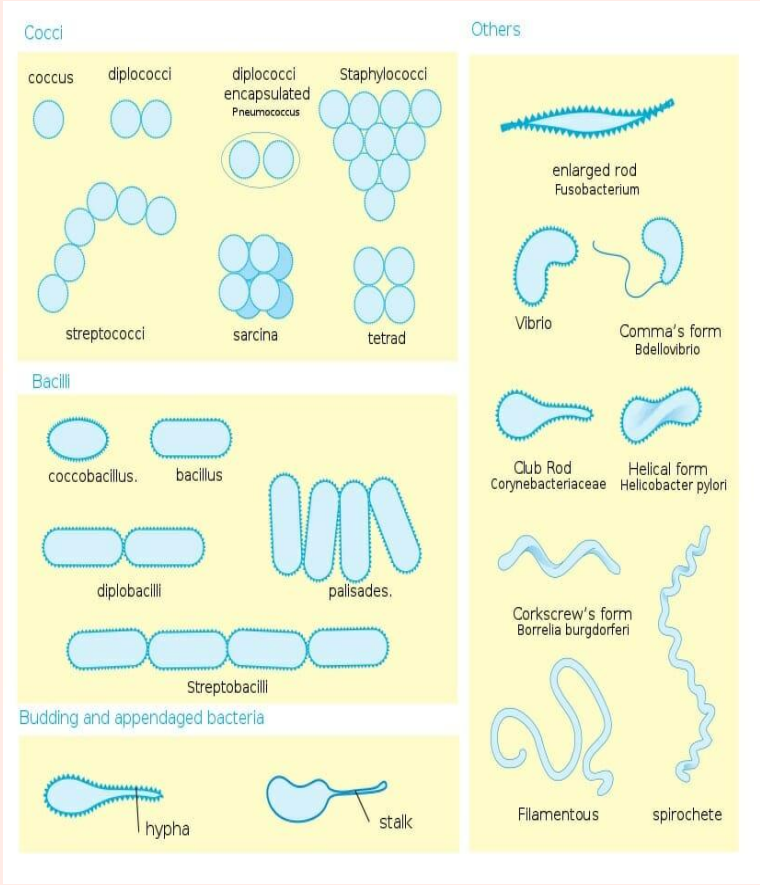
1. Spherical / Oval: Cocci
1. Rods: Bacilli
1. Very short Bacilli: Coccobacilli
1. Tapered end: Fusiform
1. Club-shaped / Curved: Vibrio
1. Helical / Spiral: Spirochaetes



Arrangements of Bacteria

Arrangements among Cocci :

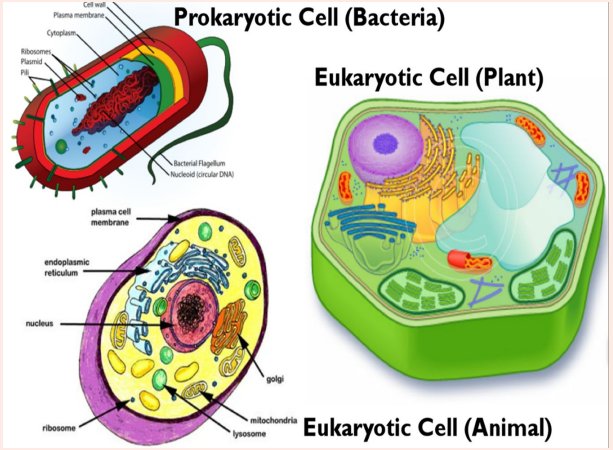
1. Pairs: Diplococci
2. Chains: Streptococci
3. Clusters: Staphylococci
4. In four: Tetrad
5. Palisades: Corynebacterium

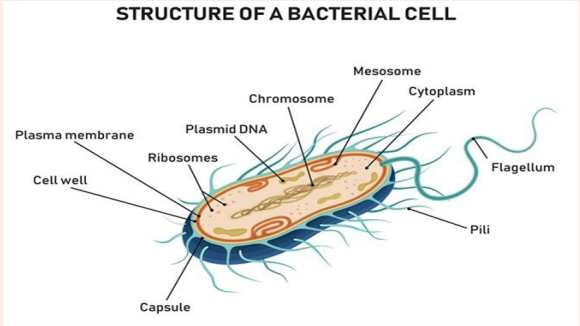


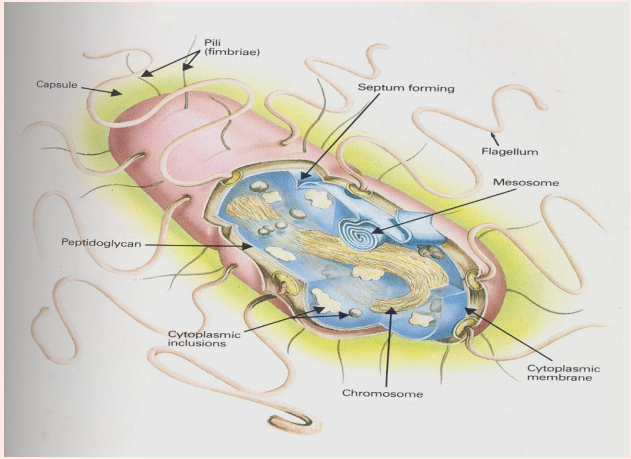
Structure of Bacteria

Dr. Note:

- The bacteria has a unique cellular structure.
- No nuclear membrane.
- No mitochondria.
- Has inclusion bodies.







Cell Wall of Bacteria

Mycoplasma have no cell wall, naturally.

- **Bacteria:** cells with rigid ***cell wall*** that ***surrounds the cytoplasmic membrane*** and internal ***structures***.

- The cell wall's chemical structure is composed of: Peptidoglycan.
  - **Peptidoglycan:** ***Rigid*** part, mucopeptide composed of alternating strands of ***N-acetyl muramic acid*** and ***N-acetyl glucosamine*** linked with ***peptide subunits***.

Dr. Note:

- The cell wall is the most important structure. It is unique from other organisms.

Bacteria cell wall functions

01 Rigidity

02 Shapes bacteria

03 Protection

04 Porous / Permeable to low molecular weight molecules.

05 Cell division

06 Antigenic determinants

Types of Bacteria

Depending on reaction to Gram Stain

Bacteria Types

Dr. Note:

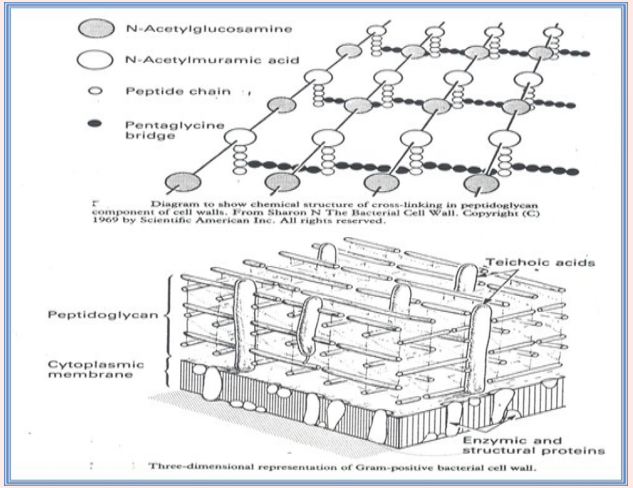
- Gram stain is named after Hans Christian Gram who discovered it.
- Gram discovered that bacteria reacts with his stain. When staining, some appear blue/purple, others appear red/pink.

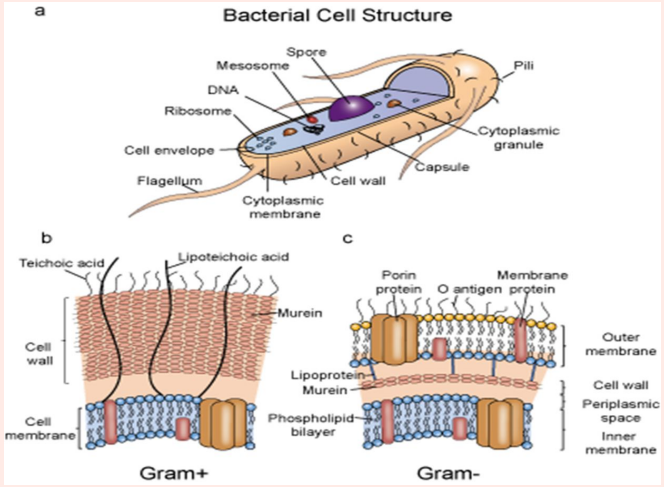
Gram Positive

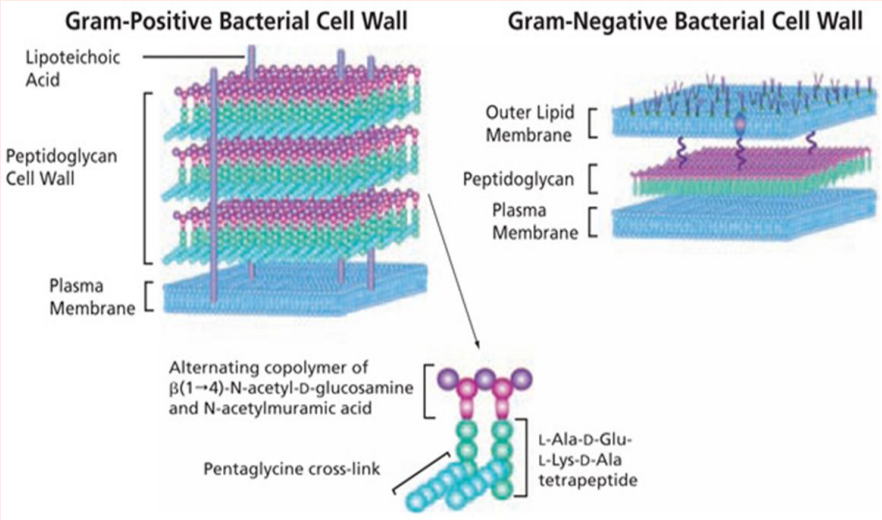
- Stain ***blue/purple*** by Gram stain (by crystal violet).
- Cell wall with ***thicker*** Peptidoglycan than gram negative.
- Cell wall Closely ***associated with*** cytoplasmic ***membrane***.
- Cell wall contains:
  1. ***Teichoic acid:***
     - Anchors cell wall to cell membrane.
     - Epithelial cell adhesion (joins bacteria with epithelial cells in humans during infections).
  2. ***Antigens*** (for identification and induction of immune system):
     - Polysaccharides (Lancefield)
     - Protein (Griffith)

Gram Negative

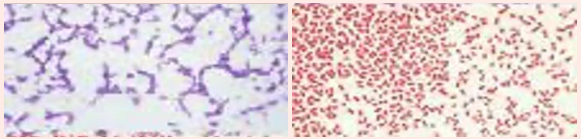
- Stain ***red*** by Gram stain (by safranin).
- Cell walls with ***thin*** Peptidoglycan.
- **Outer membrane** contains:
  1. ***Specific proteins*** (porins):
     - Important in the transport of hydrophilic molecules.
  2. ***Lipopolysaccharide*** (Endotoxin).
     - Found only in the outer membrane of gram negative bacteria.

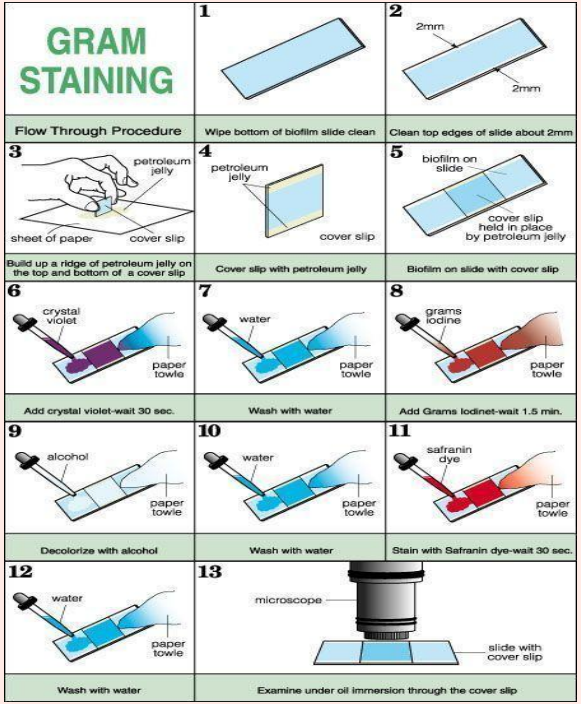

N-Acetylglucosamine
N-Acetylmuramic acid
Peptide chain
Pentaglycine bridge
Diagram to show chemical structure of cross-linking in peptidoglycan component of cell walls. From Sharon N The Bacterial Cell Wall. Copyright (C) 1969 by Scientific American Inc. All rights reserved.
Teichoic acids
Peptidoglycan
Cytoplasmic membrane
Enzymic and structural proteins
Three-dimensional representation of Gram-positive bacterial cell wall.

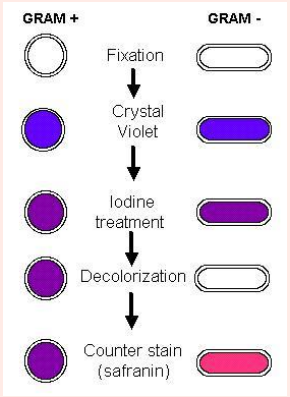

a
Bacterial Cell Structure
Spore
Mesosome
Pili
DNA
Ribosome
Cytoplasmic granule
Cell envelope
Capsule
Flagellum
Cell wall
Cytoplasmic membrane
b
Teichoic acid
Lipoteichoic acid
Murein
Cell wall
Cell membrane
Gram+
c
Porin protein
O antigen
Membrane protein
Outer membrane
Lipoprotein
Murein
Cell wall
Periplasmic space
Phospholipid bilayer
Inner membrane
Gram-

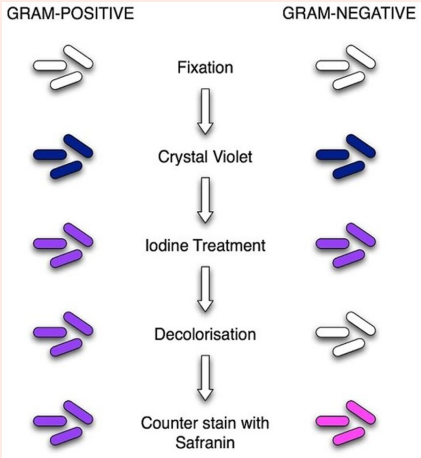

Gram-Positive Bacterial Cell Wall
Lipoteichoic Acid
Peptidoglycan Cell Wall
Plasma Membrane
Gram-Negative Bacterial Cell Wall
Outer Lipid Membrane
Peptidoglycan
Plasma Membrane
Alternating copolymer of β(1→4)-N-acetyl-D-glucosamine and N-acetylmuramic acid
Pentaglycine cross-link
L-Ala-D-Glu-L-Lys-D-Ala tetrapeptide

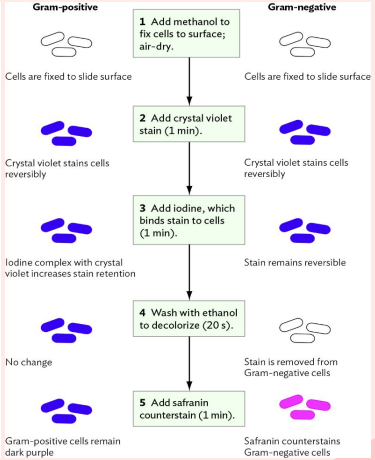
GRAM STAINING PROCESS











Dr. Note:

- The gram staining process has to be in order as in the pictures.
- After we fix the slide and make a smear, we stain it with Crystal violet.
- During staining with Crystal violet for about one minute, the peptidoglycan will take the stain.
- After washing with water, we use Iodine as mordant.
- After washing, decolorization with alcohol.
- The final stain is safranin.
- The bacteria that stains crystal violet has very thick peptidoglycan and take the violet stain (gram positive).
- Gram negative bacteria stain red (from safranin) and it doesn't take the crystal violet color (it will be washed away by decolorization or water and won't be fixed).

External Structures of Bacteria

	Flagella	Pili	Capsules & Slime layer
Definition	Helical filaments.	Fine short filaments extruding from cytoplasmic membrane.	Structures surrounding the outside of cell envelope. A layer that covers the outside of the bacteria.
Where is it found?	Gram +ve & Gram -ve bacteria	Pili found on the surface of Gram +ve & Gram -ve bacteria	Not essential for cell viability. Some strains within species produce capsules while others do not.
What is it composed of?	***Flagellin*** (protein)	***Pilin*** (protein)	Usually: polysaccharide. Some: polypeptide (protein). • Example: *Bacillus anthracis*.

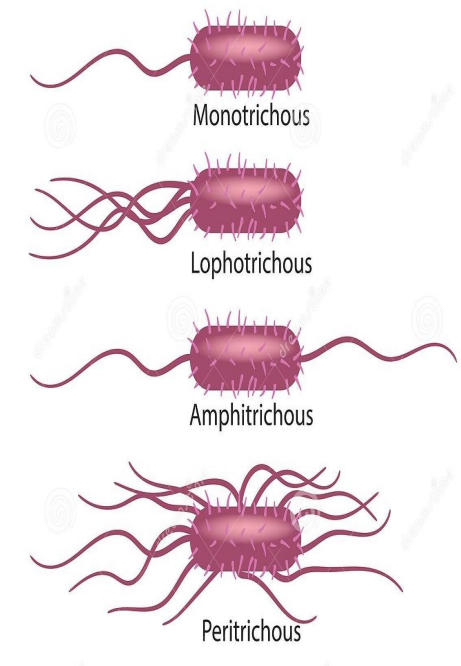
External Structures of Bacteria

	Flagella	Pili	Capsules & Slime layer
Notes	Distribution: 1. Peritrichous ○ Found mainly in gram -ve. ○ Flagella all over the surface surrounding the bacteria. 2. Monotrichous ○ Only 1 flagella. 3. Lophotrichous ○ Flagella only at the poles,. 4. Amphitrichous ○ Only one flagella at each pole.	Types: 1. Common pili (*fimbriae*): ○ Found on the surface of all gram +ve and gram -ve bacteria. 2. Sex pili: ○ Found in some bacteria only.	Can be seen by India ink also called negative stain/ special stains
Functions	1. Motility 2. Chemotaxis ○ Helps the bacteria in moving to the site of infection.	1. Common pili: ○ Adhesion & colonization. ■ Bacteria has to anchor itself to the epithelial human cell then colonizes in order to invade. 2. Sex pili: ○ Conjugation.	1. Attachment. 2. Protection from phagocytic engulfment. ○ Prevents bacteriophage and neutrophil from killing the bacteria during invasion. 3. Resistant to dryness. 4. Reservoir for certain nutrient.

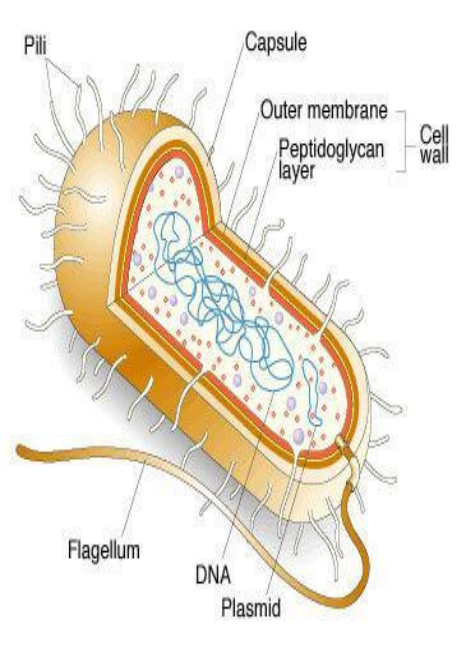
Dr. Note:

Not all Bacteria have these compositions.

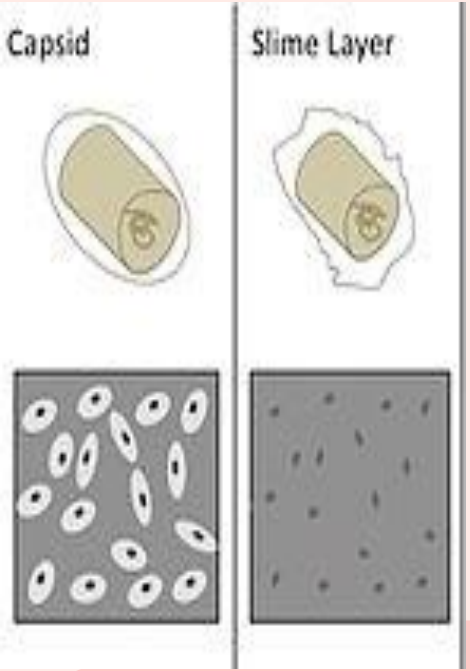
Flagella



Pili

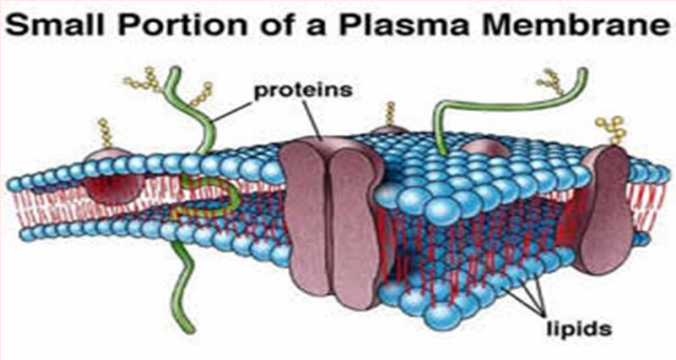


Capsules & Slime layer



Cytoplasmic Membrane (Plasma Membrane)

- **Cytoplasmic Membrane:** Double layered structure composed of phospholipid & protein.
- A ***semi-permeable*** membrane (passive diffusion).
- ***Site of*** numerous ***enzymes*** involved in ***active transport*** of nutrients and various metabolic processes.

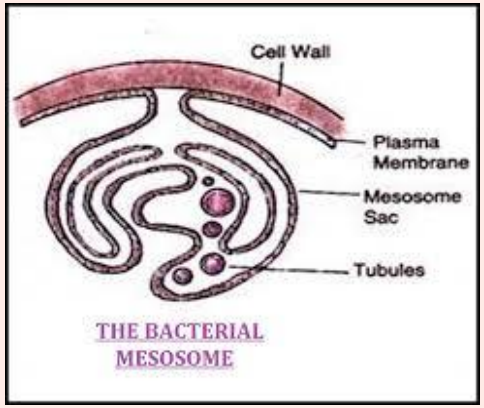


Dr. Note:

In gram positive bacteria:
cell wall → plasma membrane.

In gram negative bacteria:
Outer membrane → cell wall → plasma membrane.

Internal Structures of Bacteria



- **Mesosomes:** convolute invaginations of cytoplasmic membrane.
- Function of mesosomes:

01

Involved in DNA segregation during cell division and respiratory activity.

02

Contain receptors involved in chemotaxis.

03

Permeability barrier (active transport of solutes).

Dr. Note:

- Cytoplasmic inclusions are important for nutrition.
- Nucleoid is a primitive nucleus, not like the eukaryotic nucleus.
- Ribosomes are distributed throughout the cytoplasm, there is no Endoplasmic Reticulum in bacteria.

Core of Bacteria
(composed of)

Cytoplasmic Inclusions

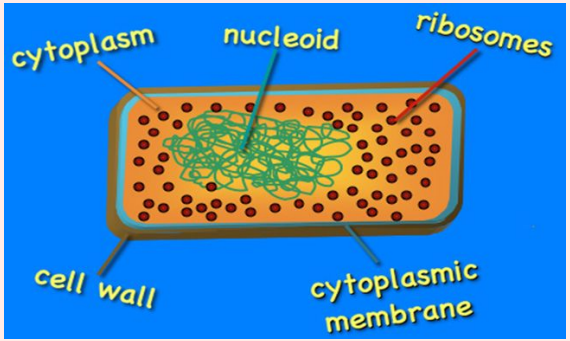
- Definition:
  - Nutritional storage granules.
- Examples:
  - Volutin
  - Lipid
  - Starch / Glycogen

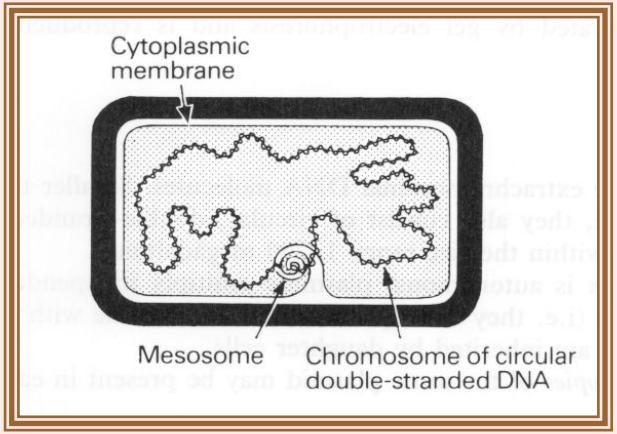
Nucleoid (Nuclear Body)

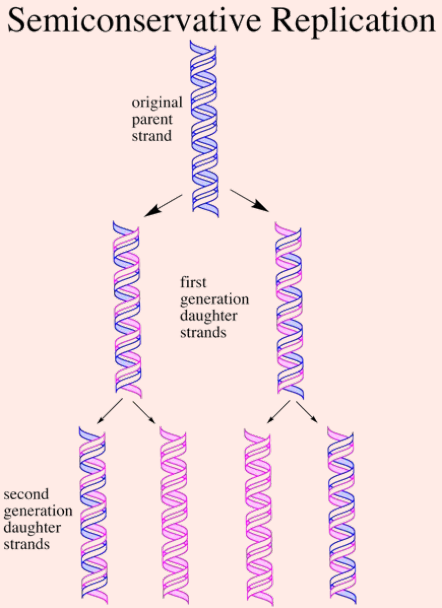
- Single (one) circular double stranded chromosome (bacteria genome / DNA)
- No nuclear membrane
- DNA undergoes semi-conservative replication
  - Bidirectional
  - From a fixed point.

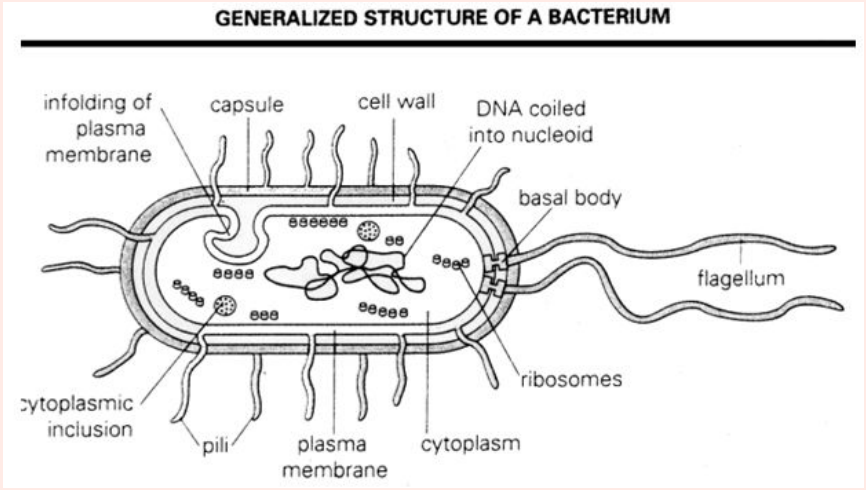
Ribosomes

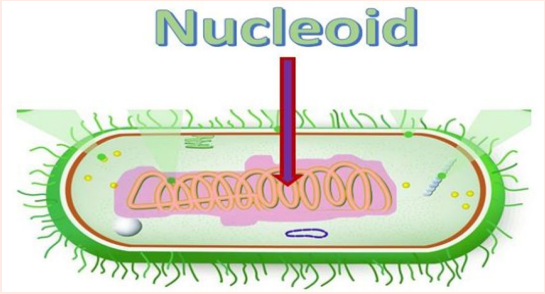
- Distributed throughout the cytoplasm.
- Site of protein synthesis.
- Composed of RNA + protein.


cytoplasm
nucleoid
ribosomes
cell wall
cytoplasmic membrane


Cytoplasmic membrane
Mesosome
Chromosome of circular double-stranded DNA


Semiconservative Replication
original parent strand
first generation daughter strands
second generation daughter strands

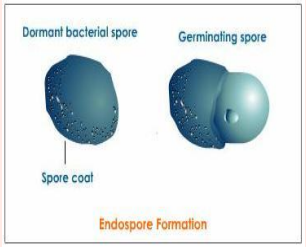

GENERALIZED STRUCTURE OF A BACTERIUM
infolding of plasma membrane
capsule
cell wall
DNA coiled into nucleoid
basal body
flagellum
ribosomes
cytoplasmic inclusion
pili
plasma membrane
cytoplasm


Nucleoid

Spores of Bacteria

Dr. Note:

Spores can survive tens of years and it will become a vegetative bacteria again once all conditions are favorable.



01

Small, dense, metabolically inactive, non-reproductive structures produced only by *Bacillus* & *Clostridium*.

02

Enables bacteria to survive adverse harsh environmental conditions.

- Resistant to:
  2. Heat
  3. Desiccation
  4. Disinfectants

+ Burns
+ chemicals

03

Germinate when growth ***conditions become favorable*** to produce vegetative cells.

04

Often remain ***associated*** with the ***cell wall***.

05

Contain high concentration of Calcium dipicolinate.

→ spores are very strong.

06

Described as :

1. Terminal
2. Sub-terminal
3. Central

Application of Spores of Bacteria

Spore preparations used for checking the efficacy of Autoclaves.

★ *Example: Bacillus subtilis & Bacillus sterothermophilus.*

Dr. Note:

- Because spores can bear heat (+100°C) and dryness, it is used in a commercial way.
- Autoclaves are used to sterilize medical equipments so that it can be used for patients.
- One way of testing the efficiency of autoclaves is inserting the spores inside the device and afterwards, into a growth media. After 5 days, if the bacteria appeared, then the devices isn't sufficient since it couldn't kill the spores.
- Spore testing is one way of testing the efficiency of autoclaves and it is done only when a new autoclave is used or once a year.

Bacterial Chromosomes

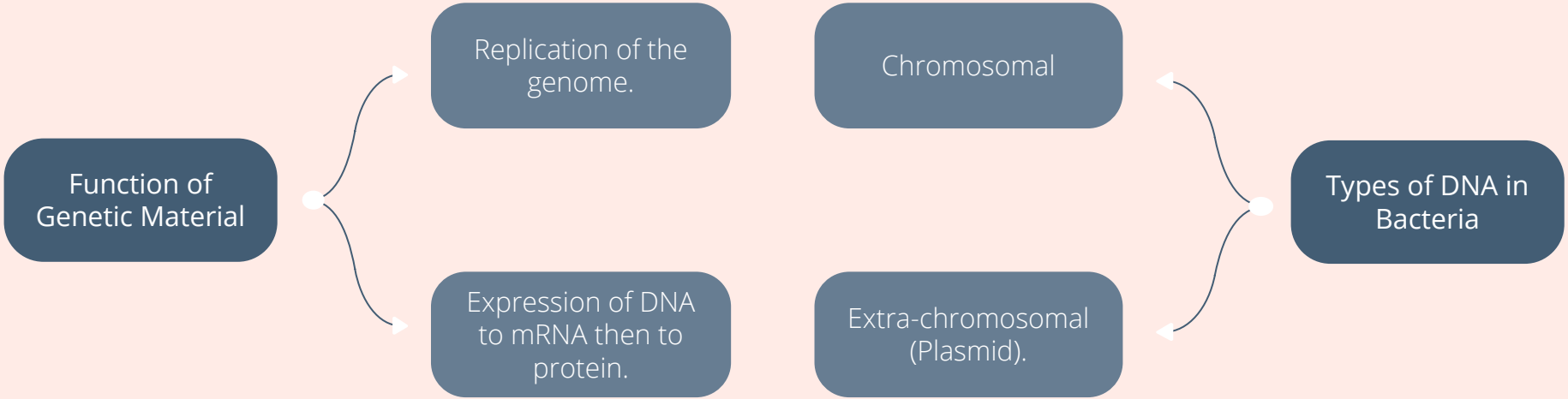
- **Chromosomes:** haploid, A single circular molecule of double stranded- DNA attached to cell membrane and located in the cytoplasm.
    - It is packed with RNA molecules and proteins to form irregular shaped structure the nucleoid (very primitive).
    - Does not have nuclear membrane.
- Genetic code in Purine (A + G) and Pyrimidine (C + T) bases of nucleotides that makes DNA strand.
- 3 bases comprise one code, each triplet codon code is for one amino acid.
- Replication is semiconservative, takes place by ***binary fission*** bacteria divides into 2 daughter cells.

Bacterial Genetics

Dr. Note:

- The plasmid is not essential for the growth of the bacteria. However, it provides unique features for the bacteria.
- The plasmid is found in the cytoplasm.

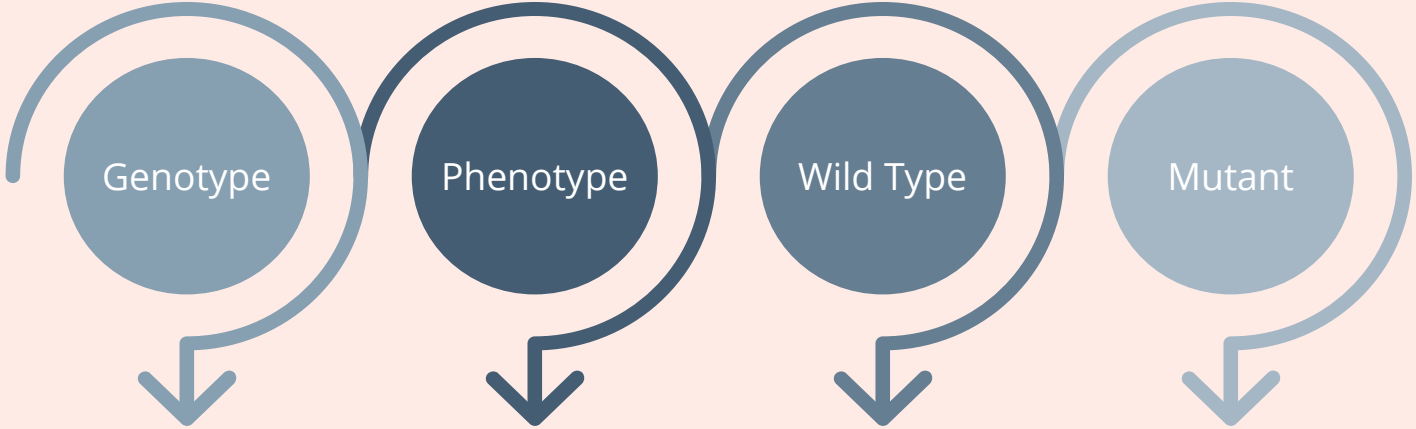
- **Genetics:** the study of inheritance and variation.
- Genetic information encoded in DNA.



Bacterial Genetics

Dr. Note:

Wild type: the original strains without any changes in the DNA or mutations.



The complete set of genetic determinants of an organism.

Expression of specific genetic material.

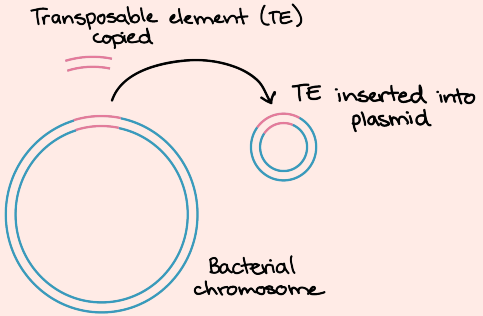
Dr. Note:
Physical characteristics.

Reference (parent) strain.

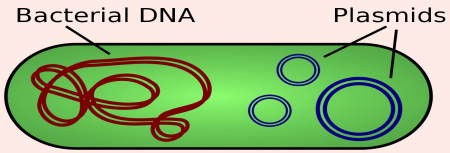
Progeny with mutation.

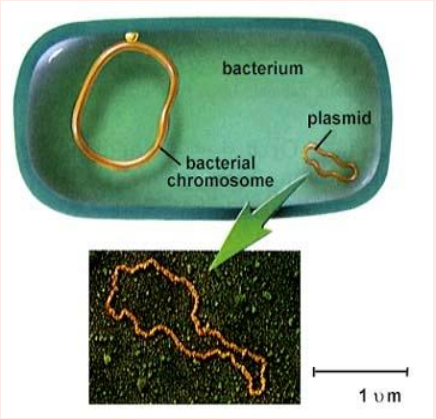
Dr. Note:
changed DNA → changed structure.

Plasmids



- **Plasmids:** Extra chromosomal DNA composed of double stranded-DNA.
- Found in: most species of bacteria.
- Origin: unknown
- Replication: govern their own replication
- Application: genetic exchange - amplify genes.
- Transfer to other bacteria by: conjugation





Dr. Note:

A segment of the DNA makes the plasmid.

Plasmids

Types of Plasmids

R-plasmids

Genes code for antibiotic resistance. Particularly Gram negative bacteria.

Col-plasmids

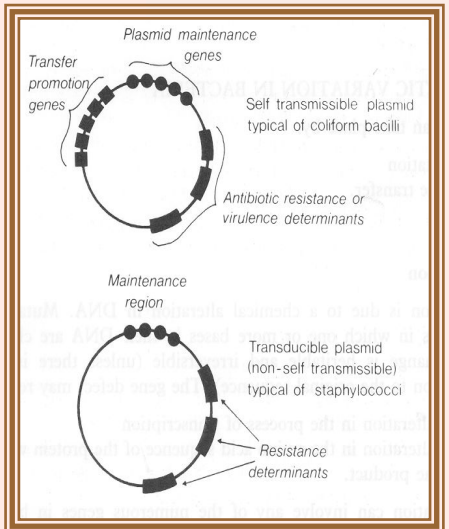
In Enterobacteria. Codes for extracellular toxins.

F-plasmids

Fertility Factor. Transfer of chromosome during mating.

Dr. Note:

- The plasmid has several genes.
- Some genes are responsible for movement.
- Other genes make the bacteria resistant to antibiotics → if a patient has an infection caused by a resistant bacteria, the patient must be isolated so that he doesn't spread the resistant gene to others.
- Some bacteria have genes that allows it to produce toxins as virulence determinants.



Dr. Note:

- A bacteria reproduces by binary fission. However, mating here is for DNA exchange.

Dr. Note:

Gene transfer: transfer of gene from a bacteria to another by various methods.
The transfer of plasmids makes bacteria resistant to antibiotics..

Genetic Variation in Bacteria

Mutations (Gene Defect)

Definition:

Inheritable changes in the structure of DNA.

Chemical changes in one or more bases of DNA.

Leads to Alternations in:

- Transcription
- Amino acid sequences
- Function

Example: Bacteria become resistant to antibiotic.

Classification:

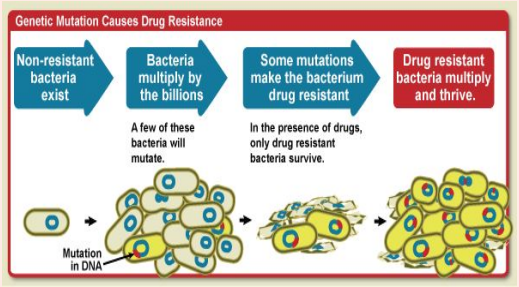
- Resistance Mutation
- Auxotrophic Mutation
- Lethal Mutation

Gene Transfer

- Transformation
- Transduction
- Conjugation

Dr. Note:

- The bacteria reproduces in the presence of mutations. Thus, the bacteria will grow in the presence of antibiotics.
- The number of mutations increase → the bacteria is now resistant to antibiotics.



Types of Mutations

Depends on biological sequencing.

Resistance Mutation

Definition:

Affects structure of cell protein.

Application in Medicine:

Bacteria become resistant to antibiotics.

Auxotrophic Mutation

Affects biosynthetic enzyme resulting in a nutritional requirement of mutant cell.

Mutant cell needs more nutritional requirements.

Lethal Mutation

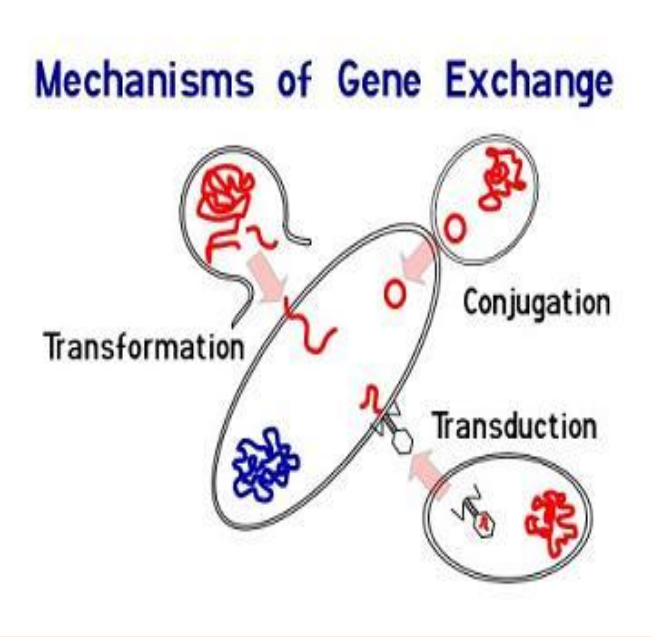
Leads to death of the bacteria.

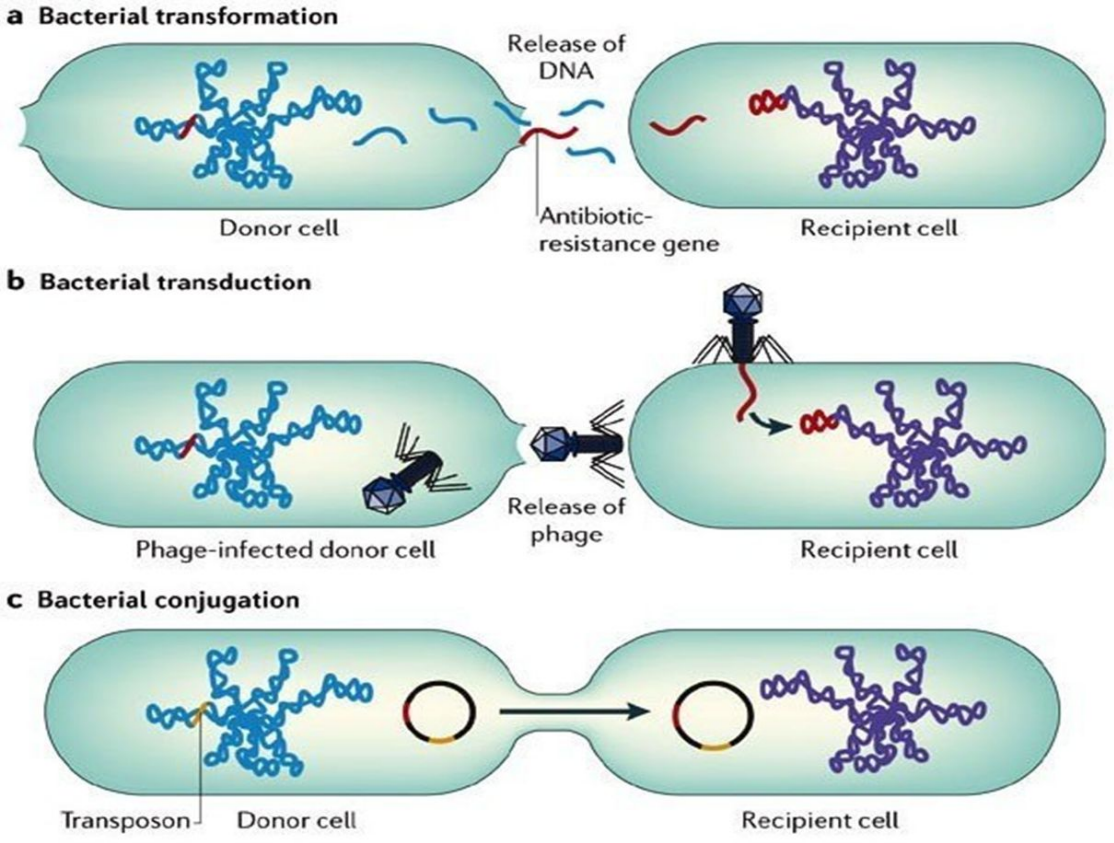
Dr. Note:

- Mutations affects transcription and transformation and amino acid sequence → changes the structure of the bacteria.
- Mutations changes the structure of some proteins in the bacteria → the bacteria becomes resistant to antibiotics.

Mechanisms of Gene Transfer Among Bacteria

	Transformation	Transduction	Conjugation
Definition	A fragment of exogenous naked bacterial DNA are taken up and absorbed into recipient cells → give it resistance features.	Phage mediated transfer of genetic information from donor to recipient cells.	-Cell contact required and genes reside on plasmid resident within donor cells transfer to recipient cell (mating). -Segments of the DNA which contain the resistance genes are transferred through plasmids. -The plasmid is transferred from a bacteria to another when the two cells are in contact through sex pilli that make a bride between two cells.
Common in	•*Haemophilus influenzae* •*Streptococcus pneumoniae*.		
Examples		1. *Staphylococcus aureus* become resistant to penicillin → secretes the enzyme Beta–Lactamase → destroys beta-lactam of penicillin. 2. *Corynebacterium diphtheriae* → produces toxin. ○ It causes a dangerous disease when secreting a toxin. The bacteria can't secrete the toxin without the gene that it gets through phage.	
	Bacteria become resistant to Ampicillin.	- The nuclear material that has the genes coding for antibiotic resistance is transferred from a bacteria to another by the phage (a virus that	- Major way bacteria acquire additional genes. - Plasmid mediated (F-factor).


Mechanisms of Gene Exchange
Transformation
Conjugation
Transduction


a Bacterial transformation
Release of DNA
Donor cell
Antibiotic-resistance gene
Recipient cell
b Bacterial transduction
Phage-infected donor cell
Release of phage
Recipient cell
c Bacterial conjugation
Transposon
Donor cell
Recipient cell

Genetic Recombination

- After gene transfer, there are three possible fates:

01

Exogenous DNA degraded by nuclease.

Exogenous:
الجزء الغريب

02

Stabilized by circulization and become plasmid.

03

Form a partially hybrid chromosome with segment derived from each source.

Dr. Note:
- As in conjugation.

Questions & Answers

Q1) A heterogenous group of unicellular organisms ?			
A) Plasmids	B) Cell wall	C) Bacteria	D) Pili
Q2) A bacteria with helical shape ?			
A) Spirochaetes	B) Bacilli	C) Fusiform	D) Cocci
Q3) What is Pili composed of ?			
A) Pilin protein	B) polypeptide	C) Flagellin	D) polysaccharide
Q4) Double layered structure composed of phospholipid & protein ?			
A) Mesosomes	B) Staphylococci	C) Cell wall	D) Cytoplasmic Membrane
Q5) what does the GRAM+ outer membrane contains?			
A) Lipid	B) Porins	C) Teichoic acid	D) Endotoxin
Q6) One of the mechanisms of Gene Transfer Among Bacteria ?			
A) colonization	B) Conjugation	C) Transcription	D) Mutant

Questions & Answers

Q1) What is Flagella composed of?
A)
Q2) A single circular molecule of double stranded- DNA located in the cytoplasm?
A)
Q3) GRAM negative stained _____ by Gram stain.
A)
Q4)Type of Mutations leads to death of bacteria ?
A)
Q5) What does the Cell wall of bacteria composed of ?
A)

A1) Flagellin protein	A2) Bacterial Chromosomes	A3) Red	A4) Lethal mutation	A5) Peptidoglycan

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