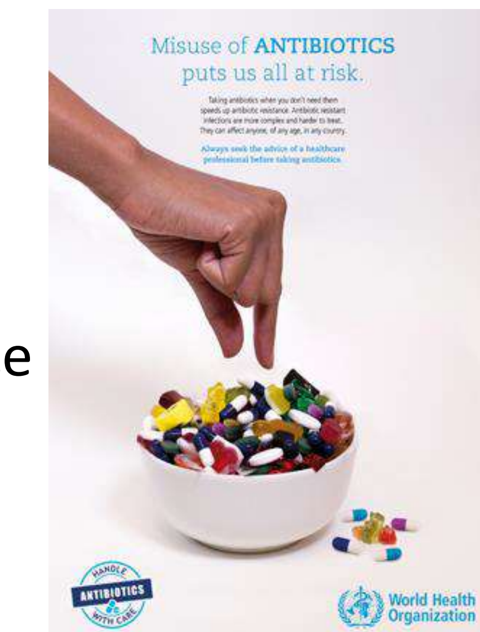
Antibacterial drugs resistance

Pharmaceutical Microbiology

Dr. Rawan Abudalo
Department of Clinical Pharmacy and Pharmacy Practice
Faculty of Pharmaceutical Sciences
Hashemite University



Introduction

- The global increase in resistance to antimicrobial drugs, including the emergence of bacterial strains that are resistant to all available antibacterial agents, has created a public health problem of potentially crisis proportions.

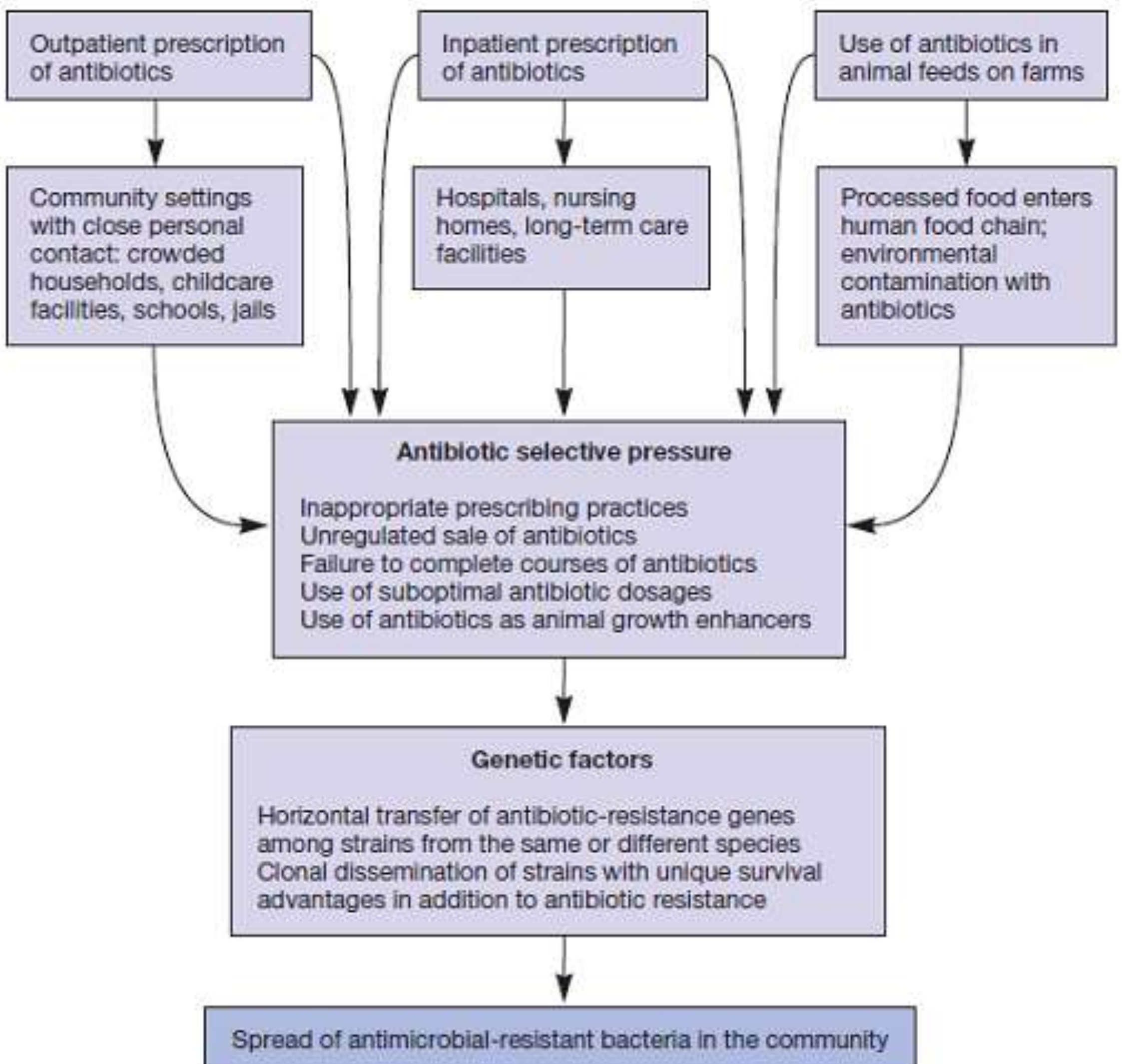
- Most pathogenic microorganisms have the capability of developing resistance to at least some antimicrobial agents.

- It is the emergence of multiple resistance, i.e. resistance to several types of antibiotic agent, that is causing major problems

Drug resistance

- Resistance represents a **CHANGE from** susceptible phenotype to a less susceptible phenotype that leads to **THERAPEUTIC FAILURE of that agent.**

Factors Affecting Antibiotic Resistance



Incomplete and indiscriminant use of antibiotics in people and animals leads to increased selective pressure on bacteria. Bacteria capable of resisting antibiotics survive and spread these traits

Usage of antibiotics

- Overuse and misuse of antibiotics by patients
- Misdiagnosis of infections and incorrect prescription
- Misuse of antibiotics in animals
- Lack of tools to monitor antibiotic resistance
- Lack of coordination between stakeholders.
- Patients should finish the prescribed course of antibiotics even when they already ‘feel better’
- Antibiotics should only be used when prescribed by a doctor and
- over-the-counter sales of antibiotics need to be closely monitored to

prevent misuse

- People should not share antibiotics with others or use leftover prescriptions

- Prevention includes:

- Reduce incidence of infection through effective hygiene and infection prevention and control .
- Appropriate Use of Antibiotics
- Use drugs combination
- Avoiding close contact with sick people
- better infection control in health care facilities
- immunisation programs

Antimicrobial resistance

Intrinsic (Natural)

Acquired

Genetic Methods

<u>Chromosomal Methods</u>

Mutations

<u>Extra chromosomal Methods</u>

Plasmids

Antimicrobial resistance

- Antimicrobial resistance can be of two

1. Intrinsic or chromosomal (always expressed in the species),

It is the innate ability of a bacterium to resist a class

of antibiotics.

- Having features such as permeability barriers, a lack of susceptibility of the cell wall, or ribosomal targets or enzymes production that make them inherently insusceptible

Antimicrobial resistance

2. Acquired resistance

A species may initially be susceptible to an antibiotic, but subsequently develop resistance. Such acquired resistance may be due to a genetic mutation within that organism, or may be derived from another organism by the acquisition of new genes.

- a. **Mutations**

Happened in structural or regulatory genes can confer resistance

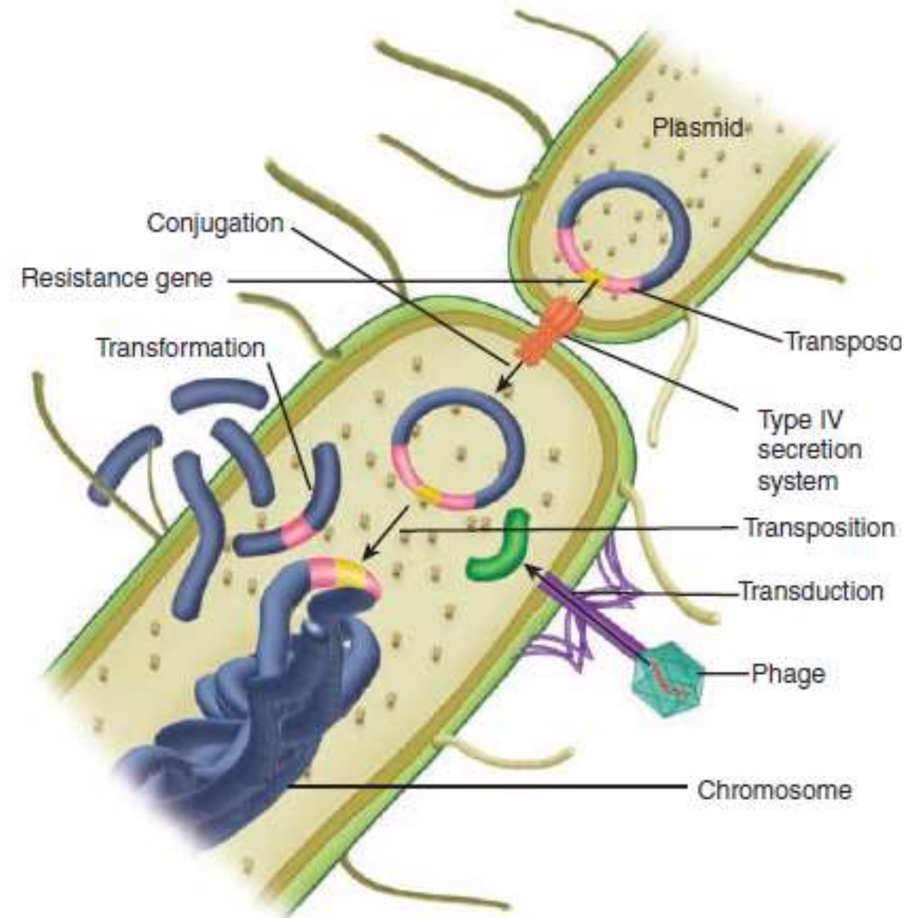
Antimicrobial resistance

b. Genetic Exchange

- four major mechanisms of genetic exchange among bacteria.

1. **transformation,**
2. **transduction,**
3. **conjugation,**
4. **Transposition.**

- **Conjugation and transposition are the most important clinically and often work in tandem.**



Plasmid-Mediated Resistance

- **Resistance plasmids (resistance factors, R factors) are**

extrachromosomal, circular, double-stranded DNA molecules that carry the genes for a variety of enzymes that can degrade antibiotics and modify membrane transport systems.

- The transfer of plasmids by conjugation was the first discovered mechanism for the acquisition of new resistance genes, and it continues to be the most important.

Transfer of r-genes from one bacterium to another

- **Conjugation : Main mechanism for spread of resistance**

The conjugative plasmids make a connecting tube between the 2 bacteria through which plasmid itself can pass.

- **Transduction : Less common method**

The plasmid DNA enclosed in a **bacteriophage is** transferred to another bacterium of same species.

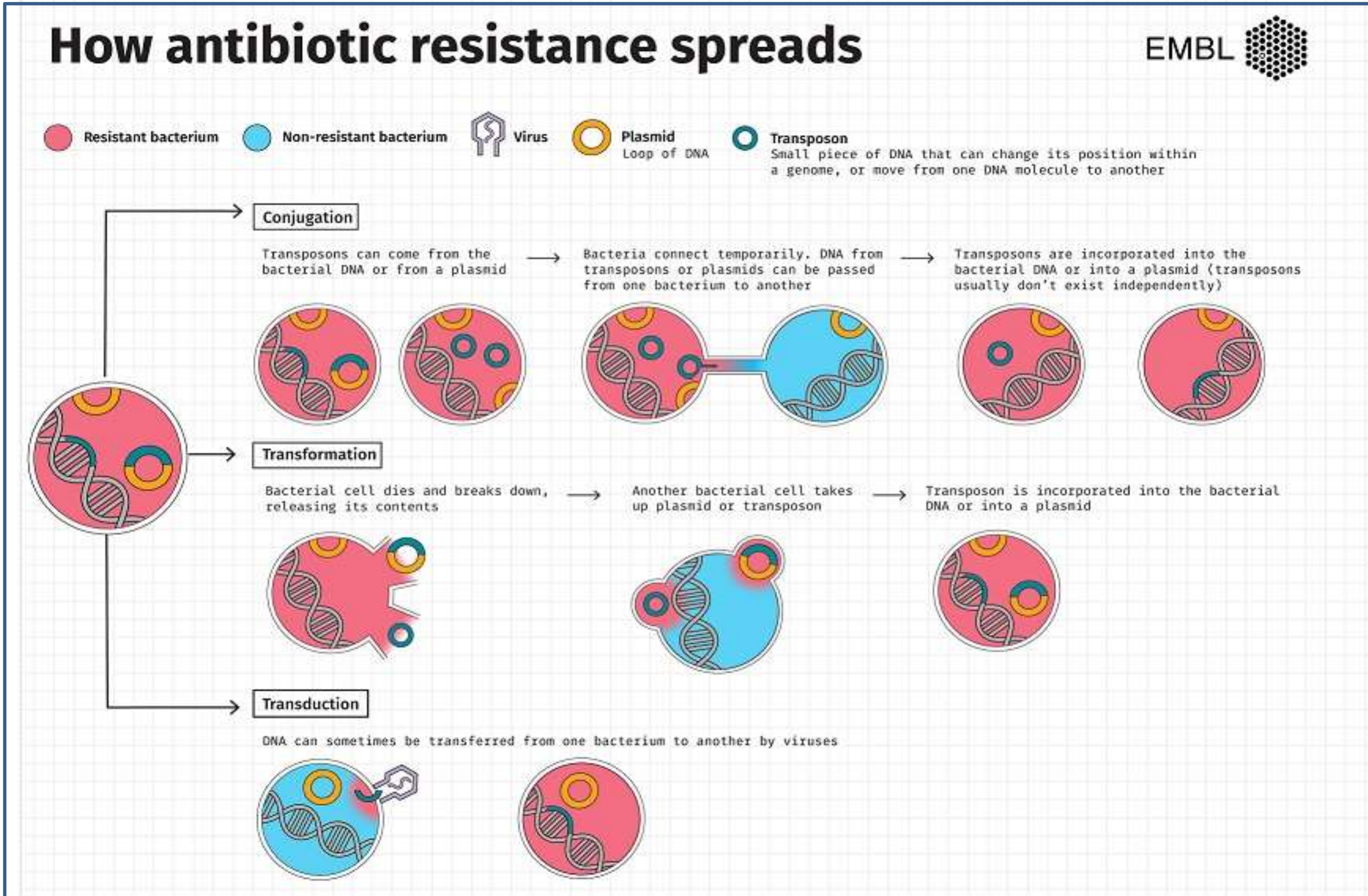
- **Transformation : least clinical problem.**
- Free DNA is picked up from the environment (i.e.. From a cell belonging to closely related or same strain

Transposons and Transposition

- Transposons containing resistance genes can move from plasmid to plasmid or between plasmid and chromosome.

- Most of the resistance genes carried on plasmids are transposon insertions that can be carried along with the rest of the plasmid genome to another strain by conjugation .
- Transposons are sequences of DNA that can move around different positions within the genome of single cell.

- The donor plasmid containing the Transposons, co-integrate with acceptor plasmid. They can replicate during co-integration


How antibiotic resistance spreads
EMBL
Resistant bacterium
Non-resistant bacterium
Virus
Plasmid
Loop of DNA
Transposon
Small piece of DNA that can change its position within a genome, or move from one DNA molecule to another
Conjugation
Transposons can come from the bacterial DNA or from a plasmid
Bacteria connect temporarily. DNA from transposons or plasmids can be passed from one bacterium to another
Transposons are incorporated into the bacterial DNA or into a plasmid (transposons usually don't exist independently)
Transformation
Bacterial cell dies and breaks down, releasing its contents
Another bacterial cell takes up plasmid or transposon
Transposon is incorporated into the bacterial DNA or into a plasmid
Transduction
DNA can sometimes be transferred from one bacterium to another by viruses

Plasmid-Mediated Resistance

Plasmid-mediated resistance is very important from a clinical point of view for three reasons:

(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, usually by conjugation.

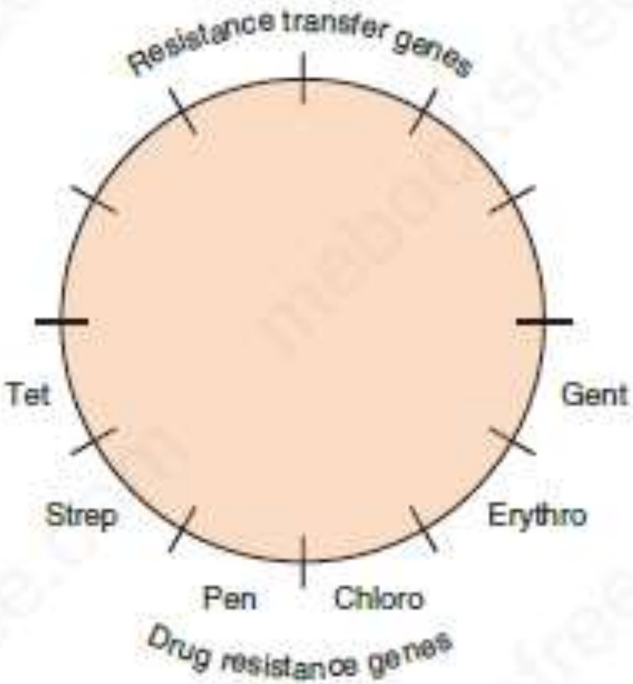


FIGURE 11–1 Resistance plasmid (R plasmid, R factor). Most resistance plasmids have two sets of genes: (1) resistance transfer genes that encode the sex pilus and other proteins that mediate transfer of the plasmid DNA during conjugation, and (2) drug resistance genes that encode the proteins that mediate drug resistance. The bottom half of the figure depicts (from left to right) the genes that encode resistance to tetracycline, streptomycin, penicillin (β-lactamase), chloramphenicol, erythromycin, and gentamicin.

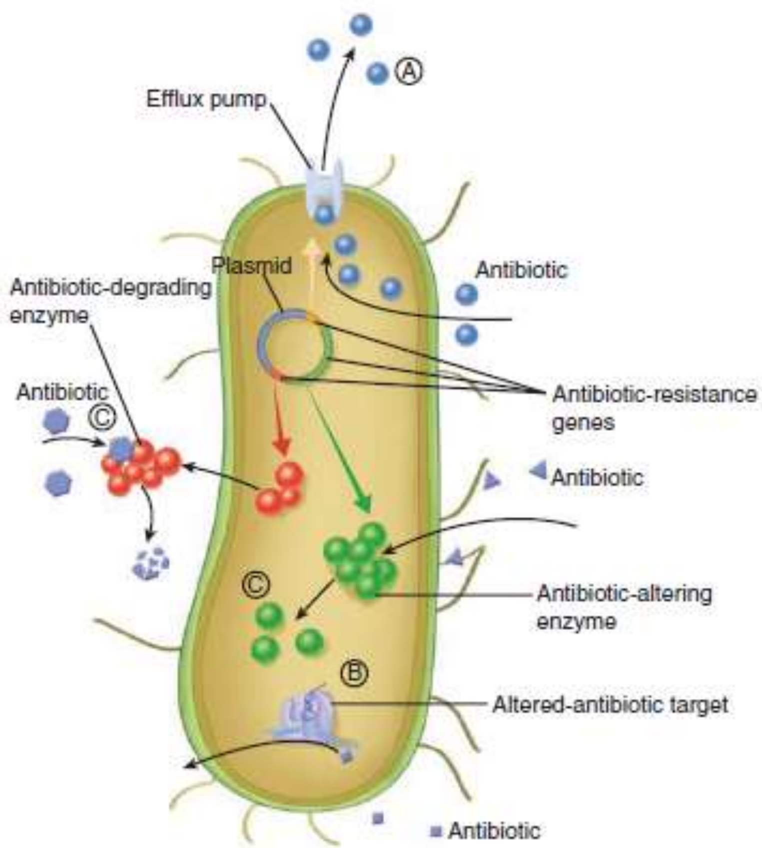
https://www.youtube.com/watch?v=n7Z5-mRB_gI

Mechanisms of resistance

- Resistance to antimicrobial agents typically occurs by one or more of the following mechanisms:

1. Inactivation of the drug
2. Alteration of the target
3. Reduced cellular uptake
4. Increased efflux

Mechanisms of resistance



Antimicrobial resistance mechanisms.

A. Exclusion barrier.
B. Altered target.
C. Enzymatic inactivation.

Mechanisms and examples of Drug Resistance

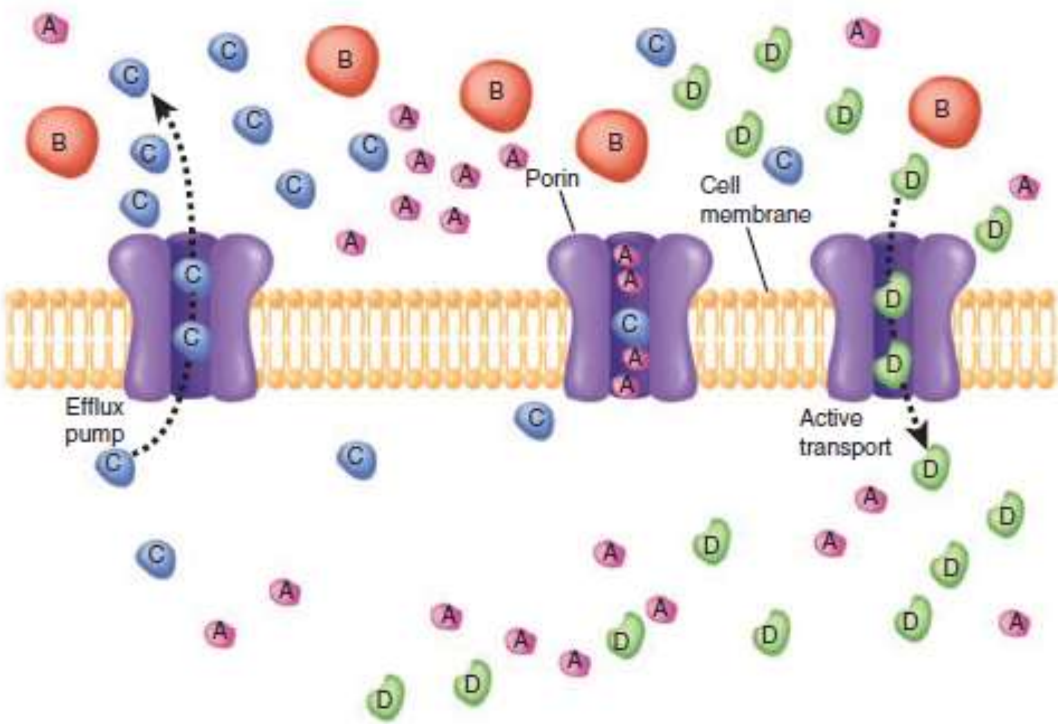
Mechanism	Important Example	Drugs Commonly Affected
Inactivate drug	Cleavage by β-lactamase	β-Lactam drugs such as penicillins, cephalosporins
Modify drug target in bacteria	1. Mutation in penicillin-binding proteins 2. Mutation in protein in 30S ribosomal subunit 3. Replace alanine with lactate in peptidoglycan 4. Mutation in DNA gyrase 5. Mutation in RNA polymerase 6. Mutation in catalase-peroxidase	Penicillins Aminoglycosides, such as streptomycin Vancomycin Quinolones Rifampin Isoniazid
Reduce permeability of drug	Mutation in porin proteins	Penicillins, aminoglycosides, and others
Export of drug from bacteria	Multidrug-resistance pump	Tetracyclines, sulfonamides, quinolones

1. *Exclusion*

- The cell wall, particularly the outer membrane, of gram-negative bacteria presents a formidable barrier for access to the interior of the cell. Inability to traverse the outer membrane is the primary reason most β-lactams are less active against gram-negative than gram-positive bacteria.

- This is a major reason for inherent resistance to antimicrobial agents, but these transport characteristics may change even in typically susceptible species due to mutations in the porin proteins

1. Exclusion



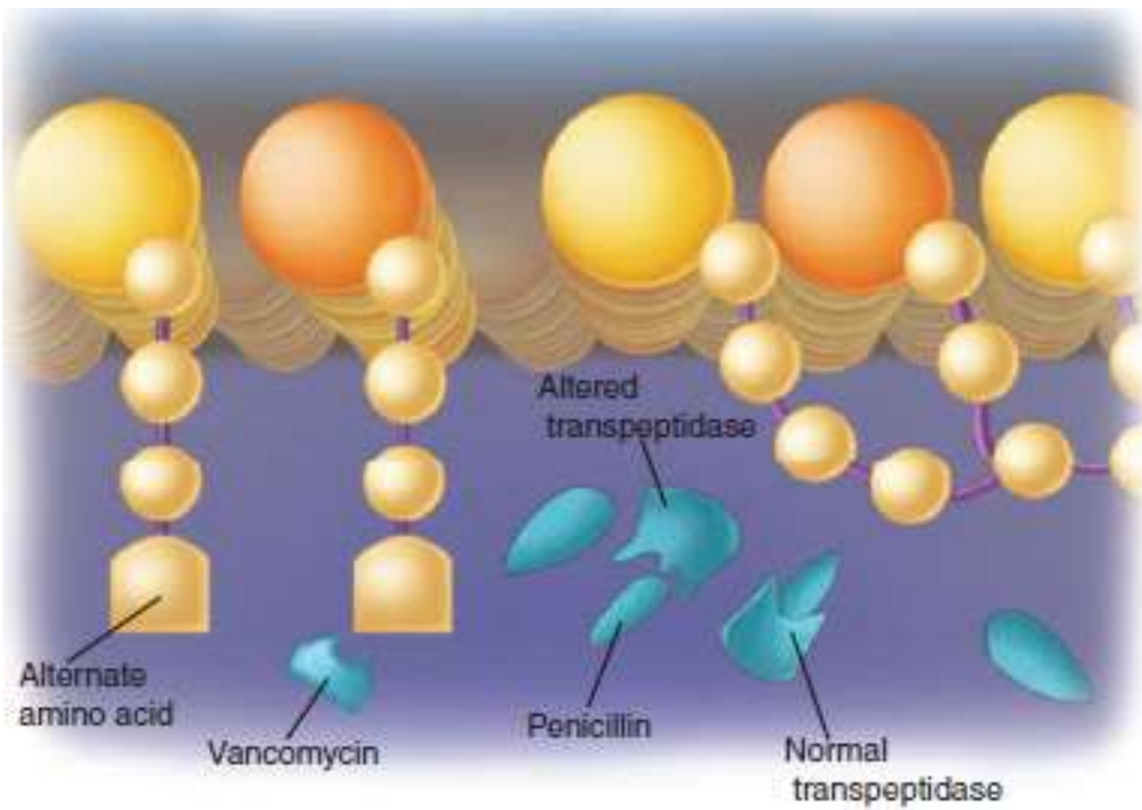
Exclusion barrier resistance. A, B, C, and D molecules are external to the cell wall here shown as what could be either the outer membrane (gram negatives) or the cytoplasmic membrane. A molecules pass through and remain inside the cell, B molecules are unable to pass due to their size, C molecules pass through but are transported back out by an efflux pump, and D molecules must be pulled through by an active process.

2. *Altered Target*

- Antimicrobials act by binding and inactivating their target, which is typically a crucial enzyme or ribosomal site. If the target is altered in a way that decreases its affinity for the antimicrobial, the inhibitory effect will be proportionately decreased.

- One of the most important examples of altered target involves the β-lactam family and the peptidoglycan transpeptidase penicillin-binding proteins (PBPs) on which they act. Changes in one or more of these proteins correlate with decreased susceptibility to multiple β-lactams.

2. *Altered Target*



A normal transpeptidase or penicillin-binding protein (PBP) is inactivated by penicillin, but penicillin no longer binds to the PBP with altered binding sites. This PBP is still able to carry out its cross-linking function so the β-lactam is no longer effective. Also shown is a terminal amino acid substitution which will no longer bind vancomycin

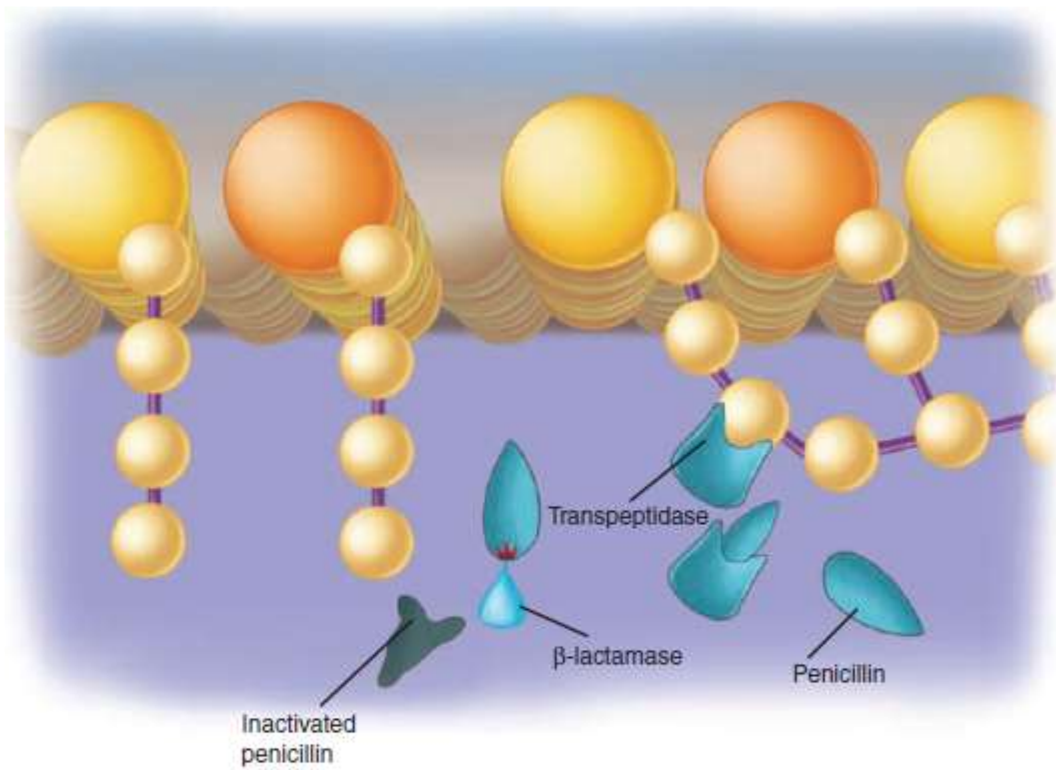
3. *Enzymatic Inactivation*

- Enzymatic inactivation of antimicrobial agents is the most powerful and robust resistance mechanism.

- β-Lactamases. β-Lactamase is a general term referring to any one of many bacterial enzymes able to break open the β-lactam ring and inactivate various members of the β-lactam group.

- Some β-lactamases are bound by the β-*lactamase inhibitor clavulanic acid, while others are not.*

3. *Enzymatic Inactivation*



The bacterium is producing a β-lactamase enzyme, which destroys penicillin by breaking open the β-lactam ring. If intact penicillin reaches a PBP, it can still bind and inactivate it; the more β-lactamase produced, the higher the level of resistance.

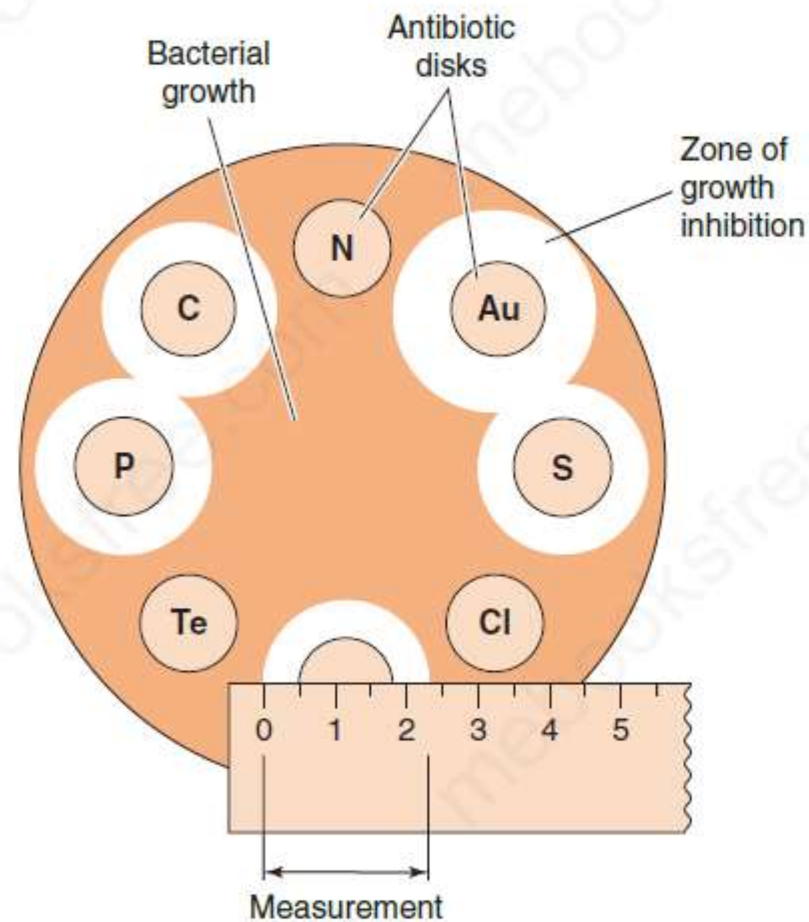
4-Modifying Enzymes

- Chemically modifying the antimicrobial molecule by bacterial enzyme to be inactive.

- The modifications take place in the cytosol or in close association with the cytoplasmic membrane .

- E.g different enzymes that acetylate, adenylate, or phosphorylate hydroxyl or amino groups on the aminoglycoside molecule.   This chemically modified aminoglycoside no longer binds to the ribosome

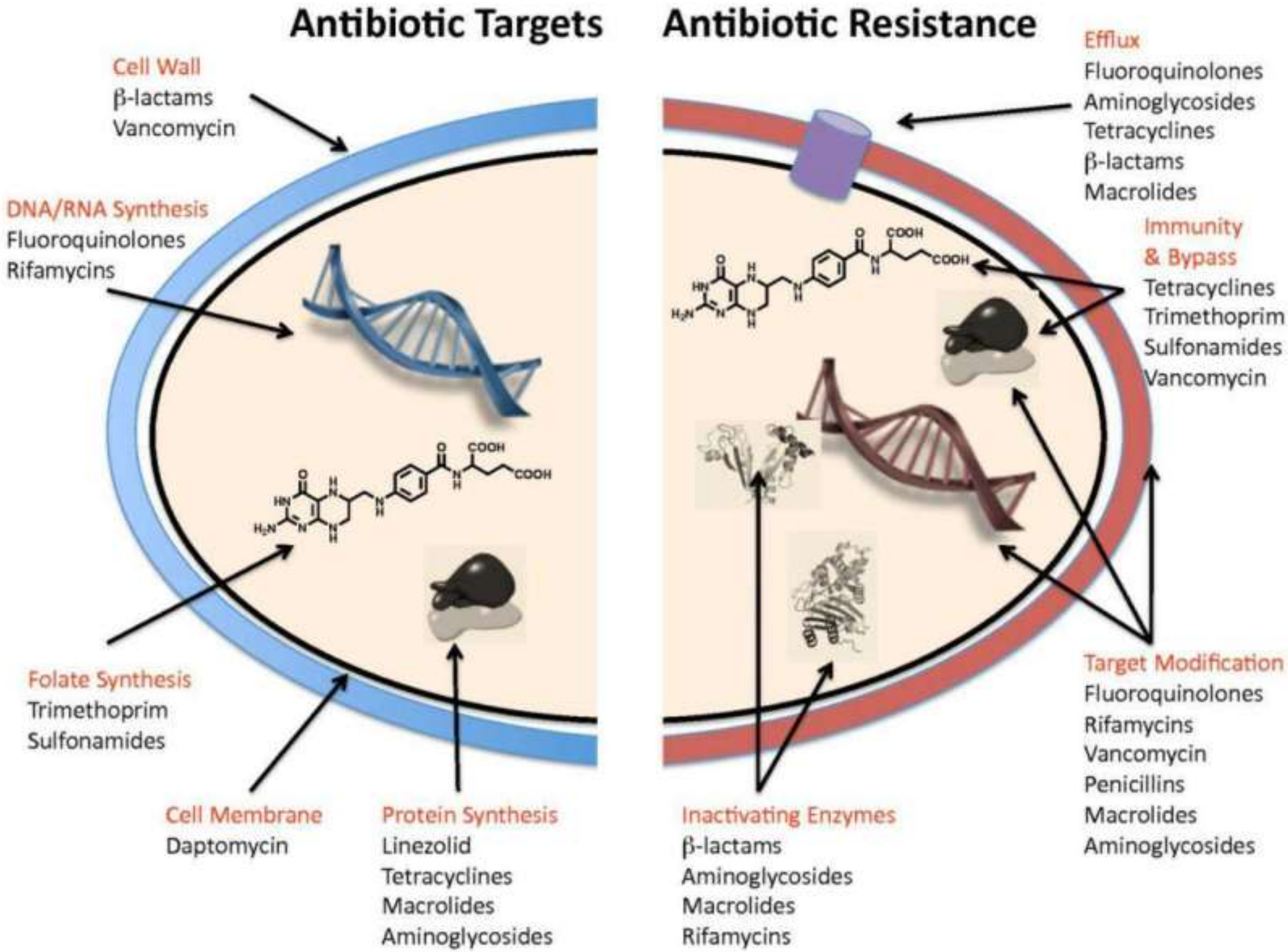
Antibiotic sensitivity testing



ANTIMICROBIAL STEWARDSHIP

The coordinated response to prevent antibiotic resistance is called "antimicrobial stewardship." Many hospitals now have formal stewardship programs, closely integrated with infection prevention teams


Enhancing infection prevention and control
Controlling the source of infection
Prescribing antibiotics when they are truly needed
Prescribing appropriate antibiotic(s) with adequate dosages
Using the shortest duration of antibiotics based on evidence
Reassessing treatment when culture results are available
Supporting surveillance of AMR and HAIs and monitoring of antibiotic consumption
Educating staff
Supporting an interdisciplinary approach
Antibiotic stewardship



Antibiotic Targets
Antibiotic Resistance
Cell Wall
β-lactams
Vancomycin
DNA/RNA Synthesis
Fluoroquinolones
Rifamycins
Folate Synthesis
Trimethoprim
Sulfonamides
Cell Membrane
Daptomycin
Protein Synthesis
Linezolid
Tetracyclines
Macrolides
Aminoglycosides
Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
β-lactams
Macrolides
Immunity
& Bypass
Tetracyclines
Trimethoprim
Sulfonamides
Vancomycin
Target Modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides
Inactivating Enzymes
β-lactams
Aminoglycosides
Macrolides
Rifamycins

Summary

A. Bacteria can become resistant to a drug by excluding it from the cell, pumping the drug out of the cell, enzymatically altering it, modifying the target enzyme or organelle to make it less drug sensitive, as examples.

B. The genes for drug resistance may be found on the bacterial chromosome or other genetic elements such as transposons

C. Chemotherapeutic agent misuse fosters the increase and spread of drug resistance, and may lead to superinfections