Name of instructor

Contact	Official email
Prof. Niveen Adel Mohamed El-wakeel	niveen10@gmail.com
Dr.Amany Elmatbouly Elsayed	amanielmatbouly@gmail.com
Dr.Aya Ahmad Elnegery	ayaelnegery@mans.edu.eg
Dr. Nada Hamid Qandeel	nadahamid@mans.edu.eg
Dr. Lamis Mohamed Taha	Lamis_mohamed@mans.edu.eg
Dr. Aya Gamal Borham	ayagamalborham@mans.edu.eg
Dr. Azza Mohamed Mamon	Azzam2010@mans.edu.eg

Contents

- Mechanism of action of antimicrobials on bacteria
- Bacterial resistance to antimicrobials

Learning outcomes

By the end of this lecture the students will be able to:

- **Describe the mechanism of action of different classes of antimicrobials.**
- **Identify causes and mechanisms of bacterial resistance.**
- **Identify different methods of antibiotic sensitivity tests**

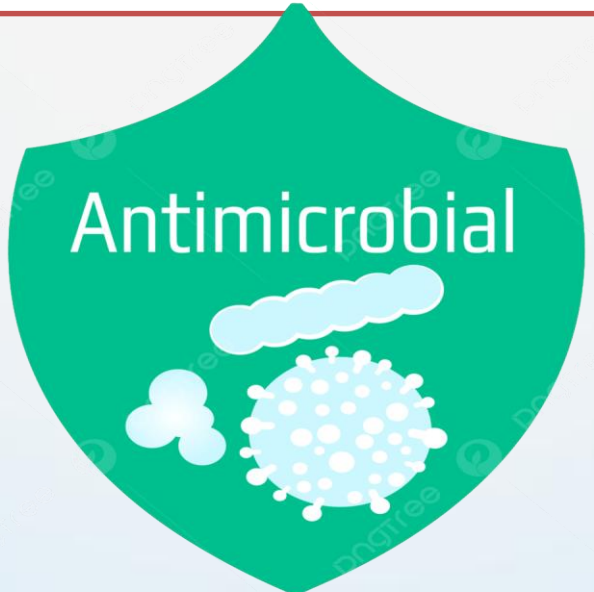
Case Senior
or Clinical correlate

26 years female complaining from UTI when seeking medical care for the first time, her doctor prescribe an antibiotic with a specified dose, but signs and symptoms doesn't subside and when seeking medical advice again the case become complicated by severe pyelonephritis and urine retention.

- **What are the possible reasons for the failure of the initial antibiotic therapy in this patient?**
- **What are the common mechanisms by which bacteria develop resistance to antibiotics?**

Antimicrobial agents

Is a chemical substance derived from a biological source or produced by chemical synthesis that kills or inhibits the growth of microorganisms.



Antibiotic

Is a naturally occurring or synthetically derived organic compound that inhibit or destroy selective bacteria.

Antimicrobial drugs

Antibacterial drugs (antibiotics)

Antiviral drugs

Antifungal drugs

Antibacterial Agents

Mechanism of action: (4 main mechanisms)

- **Cell wall synthesis.**
- **Cell membrane function.**
- **Protein synthesis.**
- **DNA replication.**

Mechanism of Action	Antibiotic Classes	Examples
Cell Wall Synthesis	Beta Lactams	Penicillins, Cephalosporins, Carbapenems, Monobactams
	Glycopeptides	Vancomycin
	Other	Bacitracin
Cell Membrane	Polymyxins	Polymyxin B, Colistin
Folate Synthesis	Sulfonamides, Trimethoprim	Sulfamethoxazole, Trimethoprim
Nucleic Acid Synthesis	DNA Gyrase Inhibitors	Quinolones
	RNA Polymerase Inhibitors	Rifampin
Protein Synthesis		
Protein Synthesis (50S)	Macrolides, Clindamycin, Linezolid, Chloramphenicol, Streptogramins	Azithromycin, Clindamycin, Linezolid
Protein Synthesis (30S)	Tetracyclines, Aminoglycosides	Doxycycline, Gentamicin

Cell Wall Synthesis

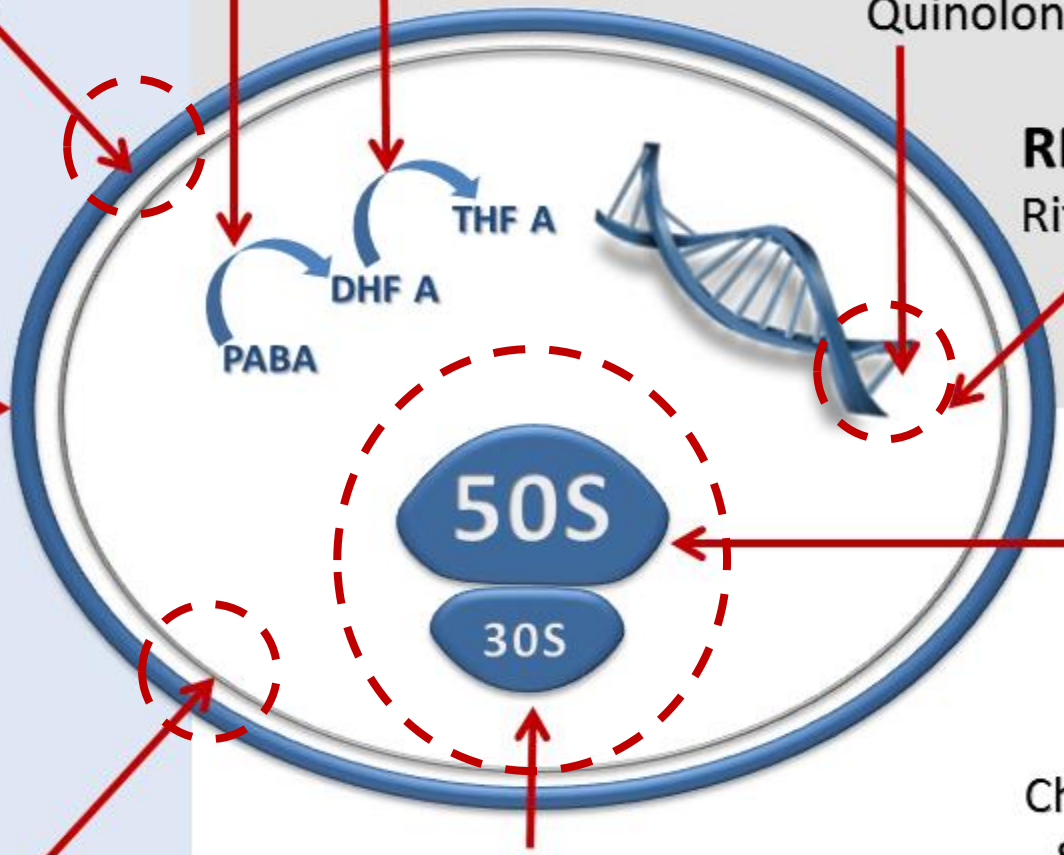
Nucleic Acid Synthesis

Folate synthesis
Sulfonamides
Trimethoprim

Beta Lactams
Penicillins
Cephalosporins
Carbapenems
Monobactams

DNA Gyrase
Quinolones

RNA Polymerase
Rifampin



Vancomycin
Bacitracin

50S subunit
Macrolides
Clindamycin
Linezolid
Chloramphenicol
Streptogramins

Cell Membrane
Polymyxins

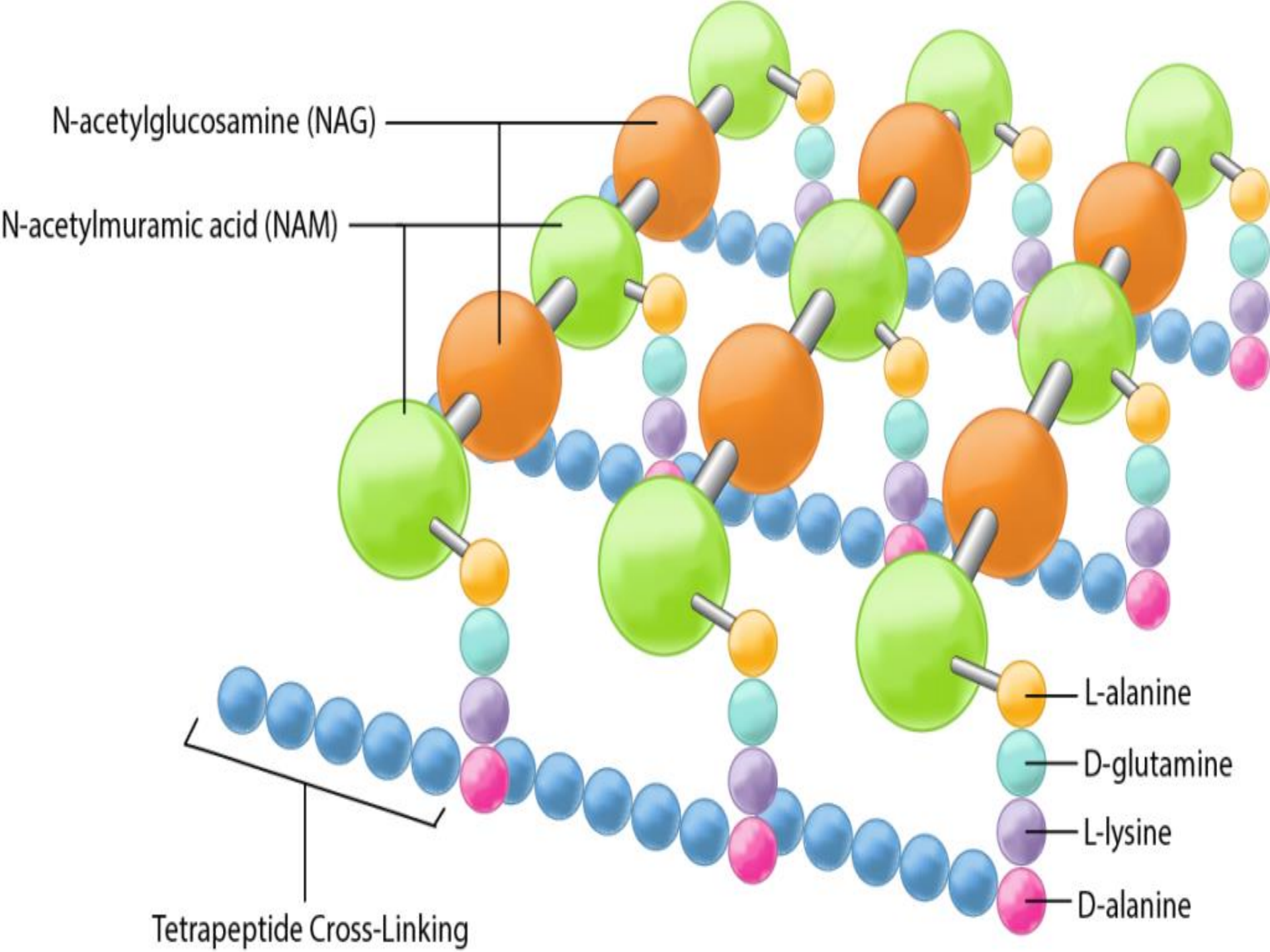
30S subunit
Tetracyclines
Aminoglycosides

Protein Synthesis

Cell wall inhibitors

Gram-Positive Cell Wall

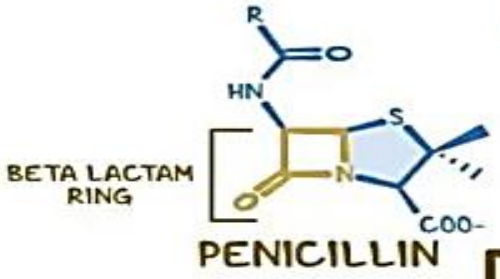
- The Gram-positive cell wall has a thick peptidoglycan layer made of **NAG** (N-acetylglucosamine) and **NAM** (N-acetylmuramic acid), connected by peptide chains.
- This layer is crucial for the bacteria's strength and shape.


N-acetylglucosamine (NAG)
N-acetylmuramic acid (NAM)
L-alanine
D-glutamine
L-lysine
D-alanine
Tetrapeptide Cross-Linking

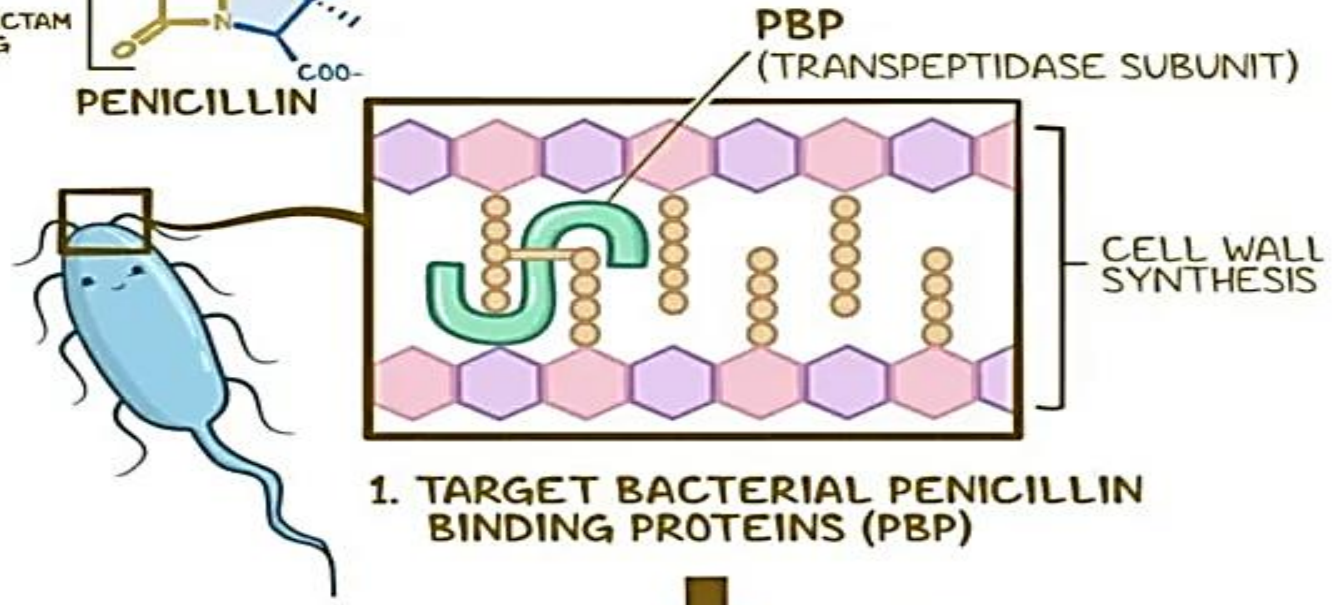
Antibiotic	Mechanism of Action	Target
Penicillins and Cephalosporins	Block enzymes (penicillin-binding proteins, PBPs) that form cross-links between NAG and NAM subunits. This weakens the peptidoglycan, causing cell lysis.	Inhibit cross-linking of peptidoglycan strands.
Bacitracin	Blocks the carrier molecule (bactoprenol) from transporting peptidoglycan precursors across the cell membrane.	Inhibit peptidoglycan precursor transport.
Vancomycin	Binds to D-Ala-D-Ala on peptidoglycan precursors, preventing their incorporation into the cell wall.	Inhibit peptidoglycan polymerization.

PENICILLIN

BETA LACTAM

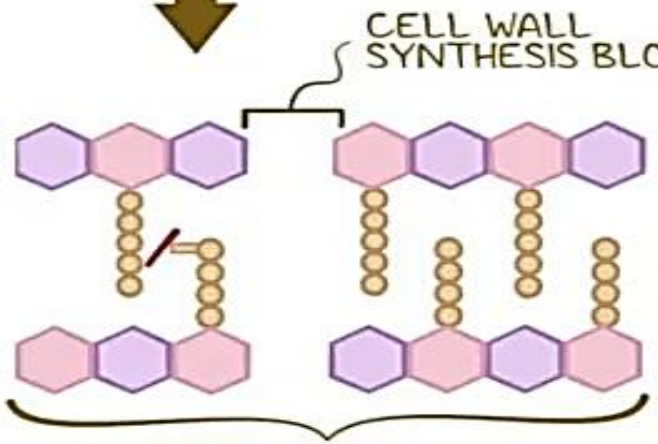


PENICILLIN

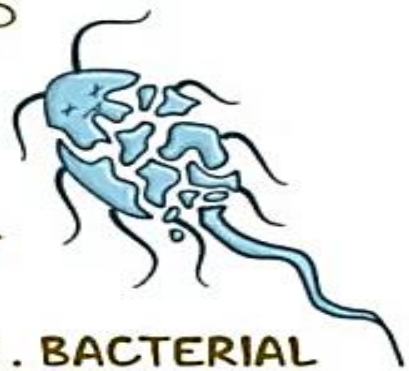


1. TARGET BACTERIAL PENICILLIN BINDING PROTEINS (PBP)

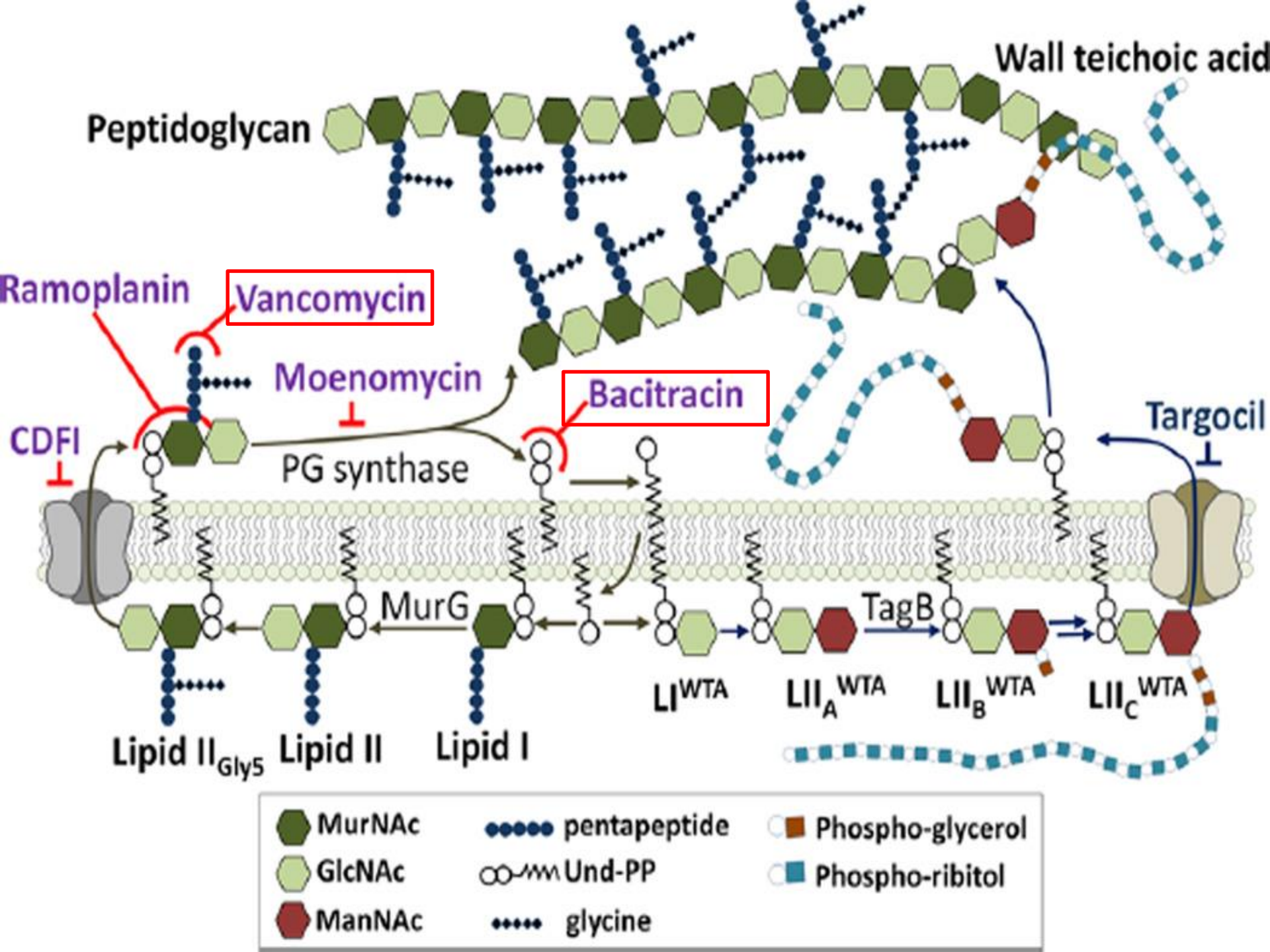
2. INTERFERE with TRANSPEPTIDATION



3. UNSTABLE CELL WALL



4. BACTERIAL DEATH


Peptidoglycan
Wall teichoic acid
Ramoplanin
Vancomycin
Moenomycin
Bacitracin
CDFI
Targocil
PG synthase
MurG
TagB
LI^WTA
LII_A^WTA
LII_B^WTA
LII_C^WTA
Lipid II_Gly5
Lipid II
Lipid I
MurNAc
GlcNAc
ManNAc
pentapeptide
Und-PP
glycine
Phospho-glycerol
Phospho-ribitol

Desired properties of antibiotics (ideal antibiotic)

1. **Selective toxicity.**
2. **Bacteriocidal rather than bacteriostatic.**
3. **Broad spectrum.**
4. **Water soluble and stable.**
5. **Long plasma half-life.**
6. **Good tissue distribution.**
7. **Non-allergic.**
8. **Do not develop antibacterial resistance.**

ANTIBIOTIC COMBINATION

- Administration of more than one antibiotic may be beneficial as well as may be harmful.
- The effect of antibiotic combination may be predictable in general terms:

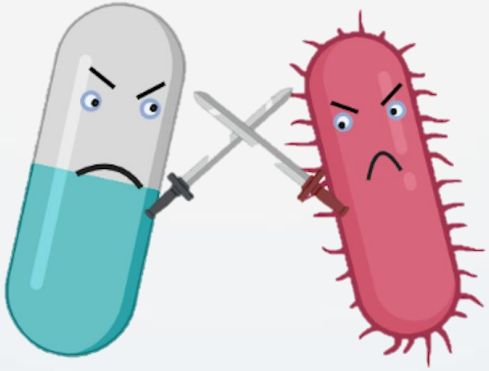
1. Synergism: A bactericidal drug when combined with another bactericidal drug may produce a synergistic effect.

2. Antagonism: A bacteriostatic drug combined with a bactericidal drug is likely to produce **antagonistic** effect.

3. Addition: A bacteriostatic drug combined with another bracteriostatic drug is usually merely additive.
Example: Cotrimoxazole a combination of trimethoprim and sulphamethoxazole.

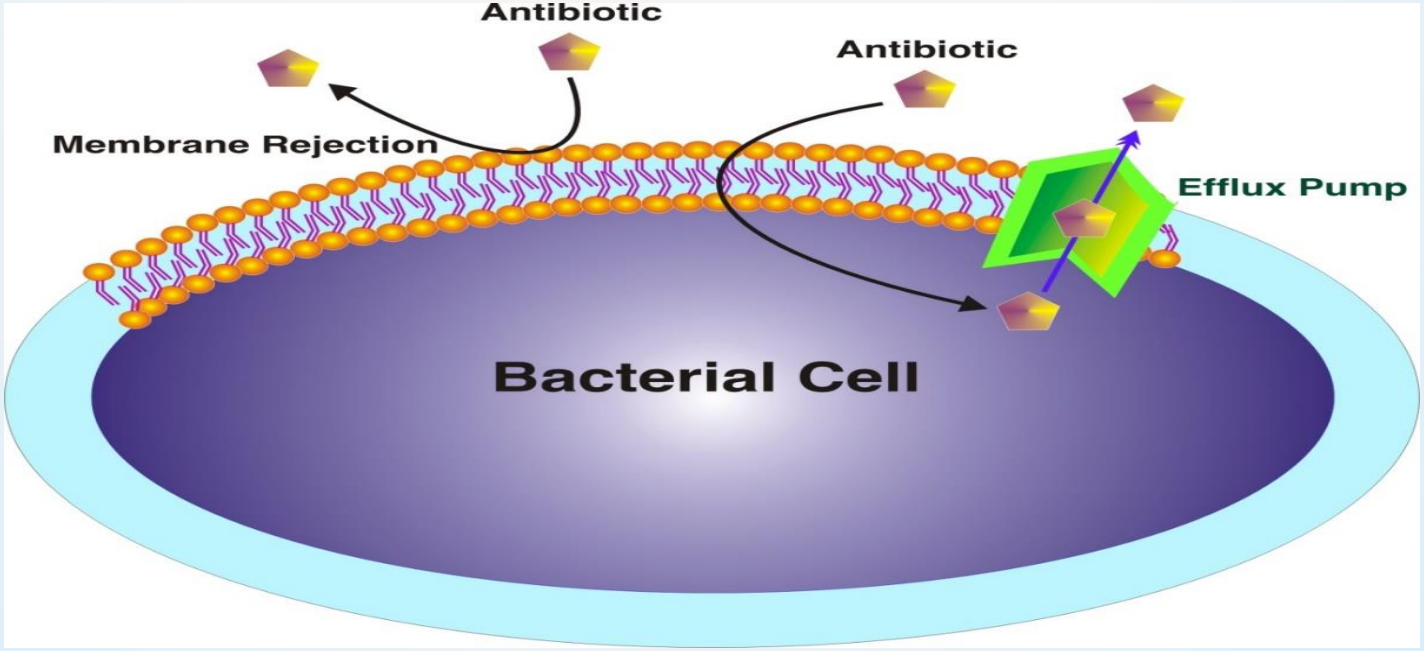
Advantages:	Disadvantages:
1. Promote treatment in patients suspected to have serious microbial infections. 2. Prevent or delay drug resistance. 3. Effective in mixed or unknown infection.	1. Increase the chance for drug reaction. 2. High cost. 3. Drug antagonism possibility

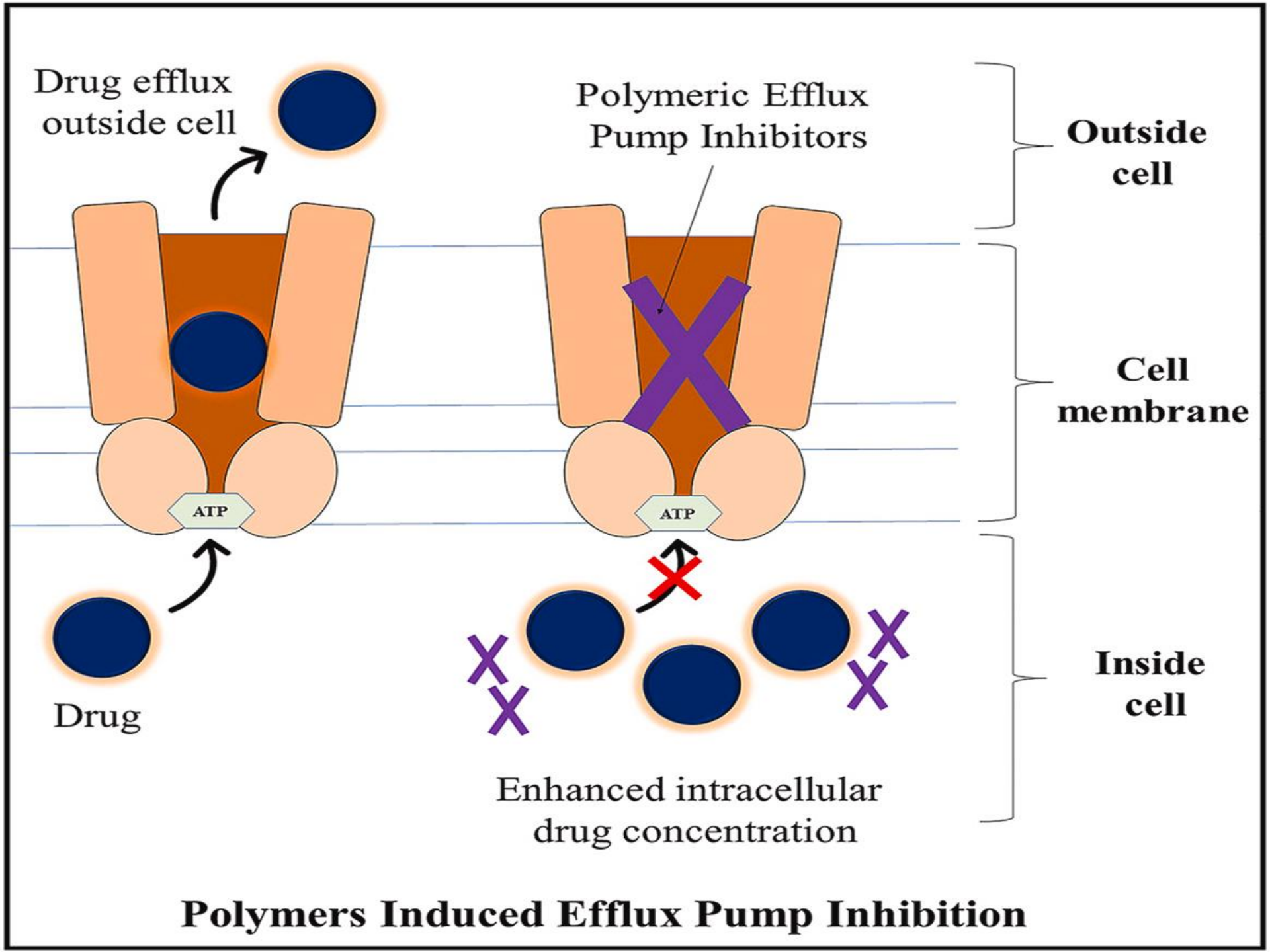
BACTERIAL RESISTANCE TO ANTIMICROBIAL DRUGS



Mechanisms of resistance

1. **Reduction of intracellular antibiotic accumulation by decreasing permeability and/or increasing active efflux of the antibiotic (efflux pump).**

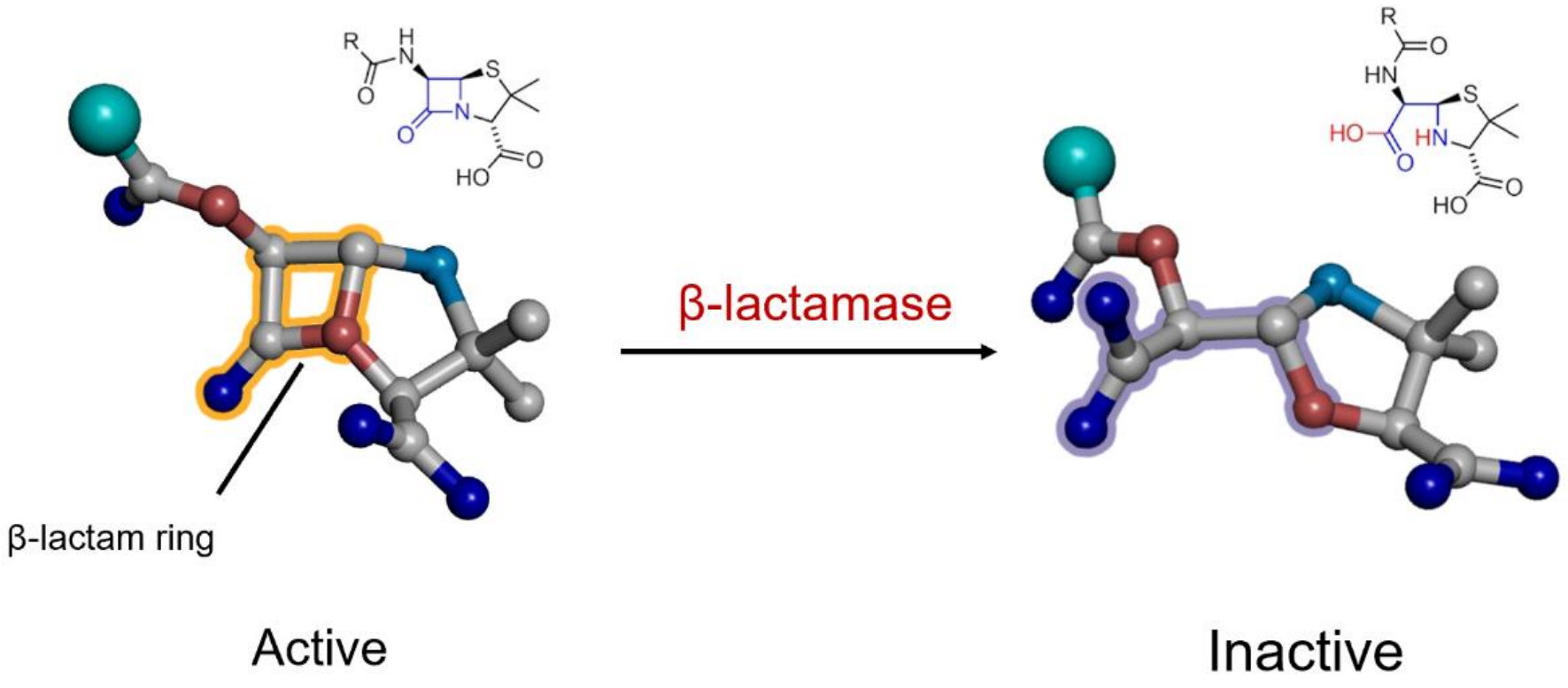




Polymers Induced Efflux Pump Inhibition

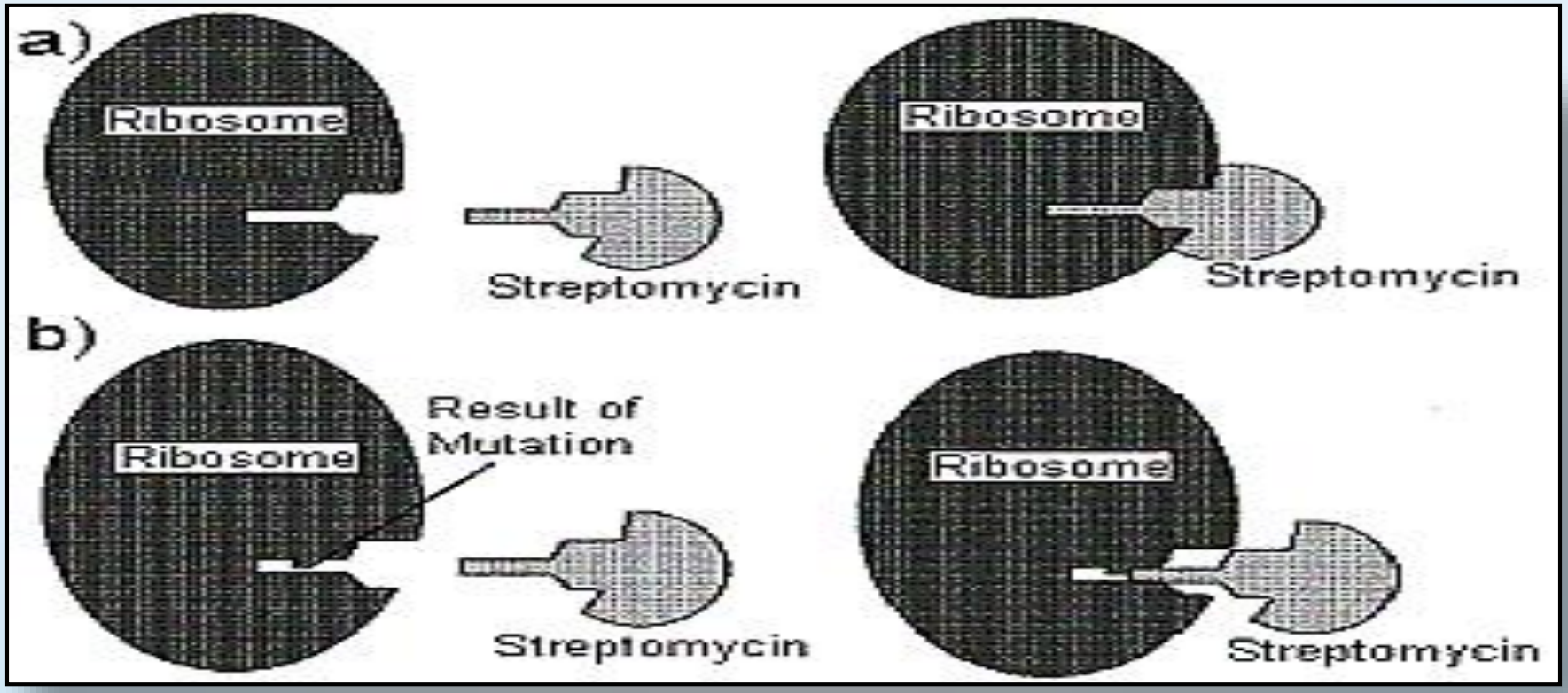
2. Enzymatic inactivation:

- β-lactamases, that inactivate β-lactams.
- Acetyl transferases that inactivate Tetracyclins.



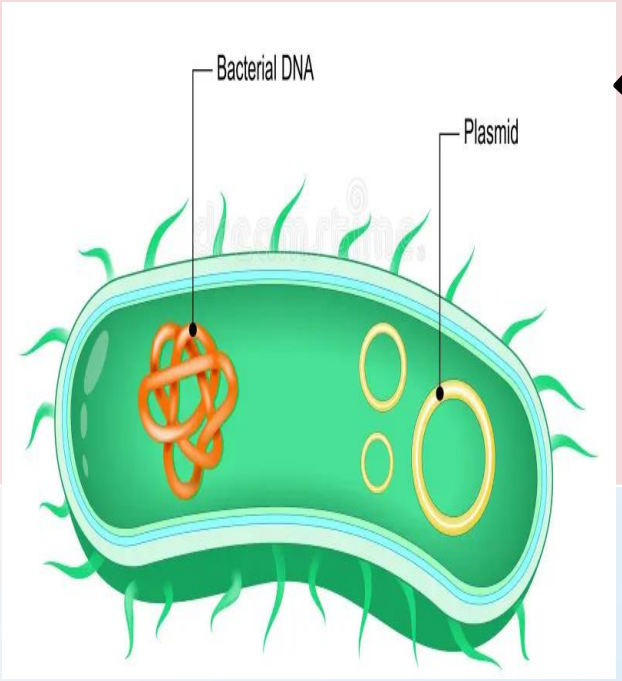
3. Alteration of target site:

- Occurs due to mutations that alter the site targeted by the antibiotic.
- **P12 of 30S ribosomal subunit → R (Streptomycin).**



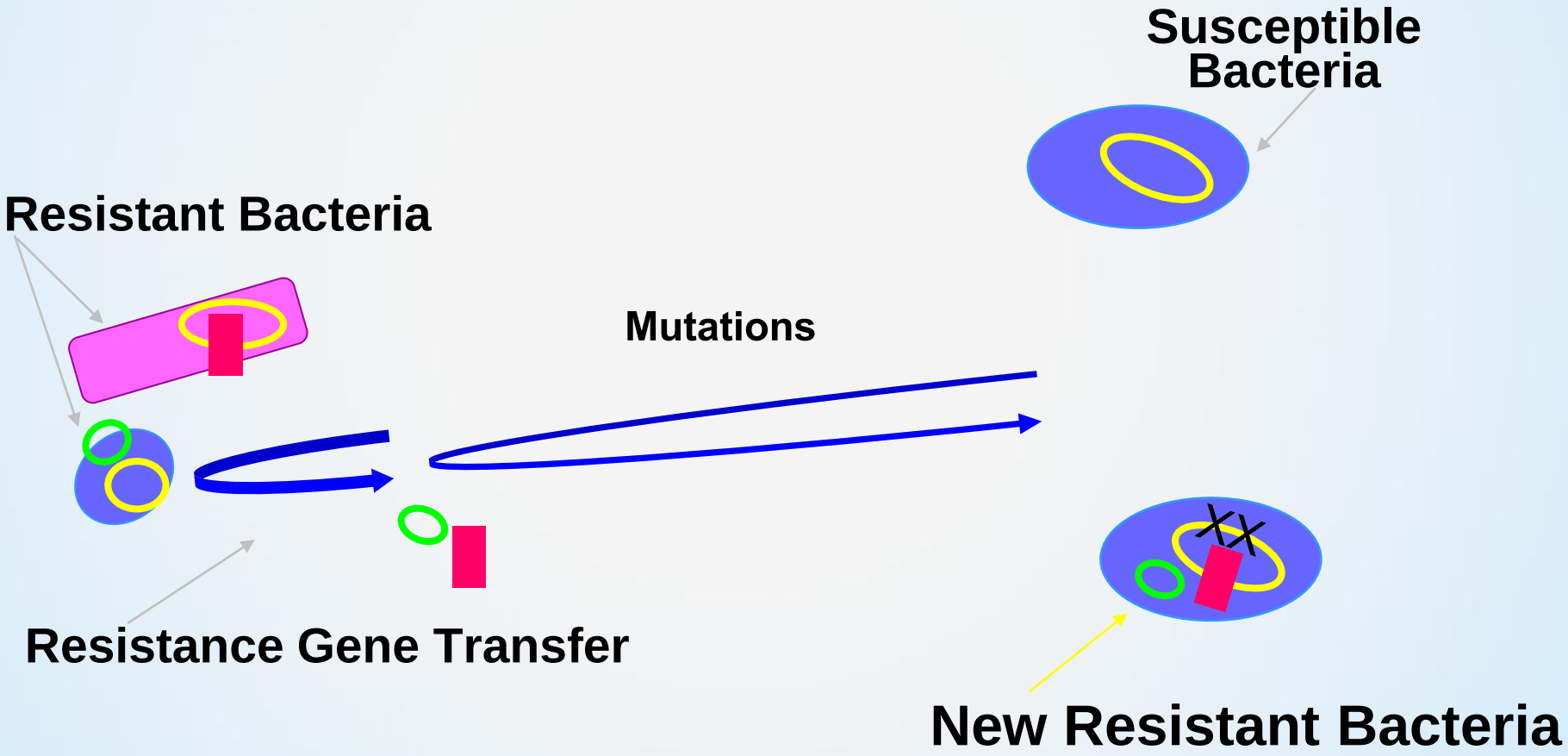
Origin of Drug Resistance

Non-genetic.	Genetic:
The organism can resist antimicrobial agents through: 1. Decreased permeability of the organism to the drug. 2. Inactivation of the drug by enzymes produced by the organism. 3. Development of an altered structural target site for the drug. 4. Development of an altered metabolic pathway. 5. Production of enzymes less affected by the drug.	**A. Chromosomal** **B. Extra chromosomal.**

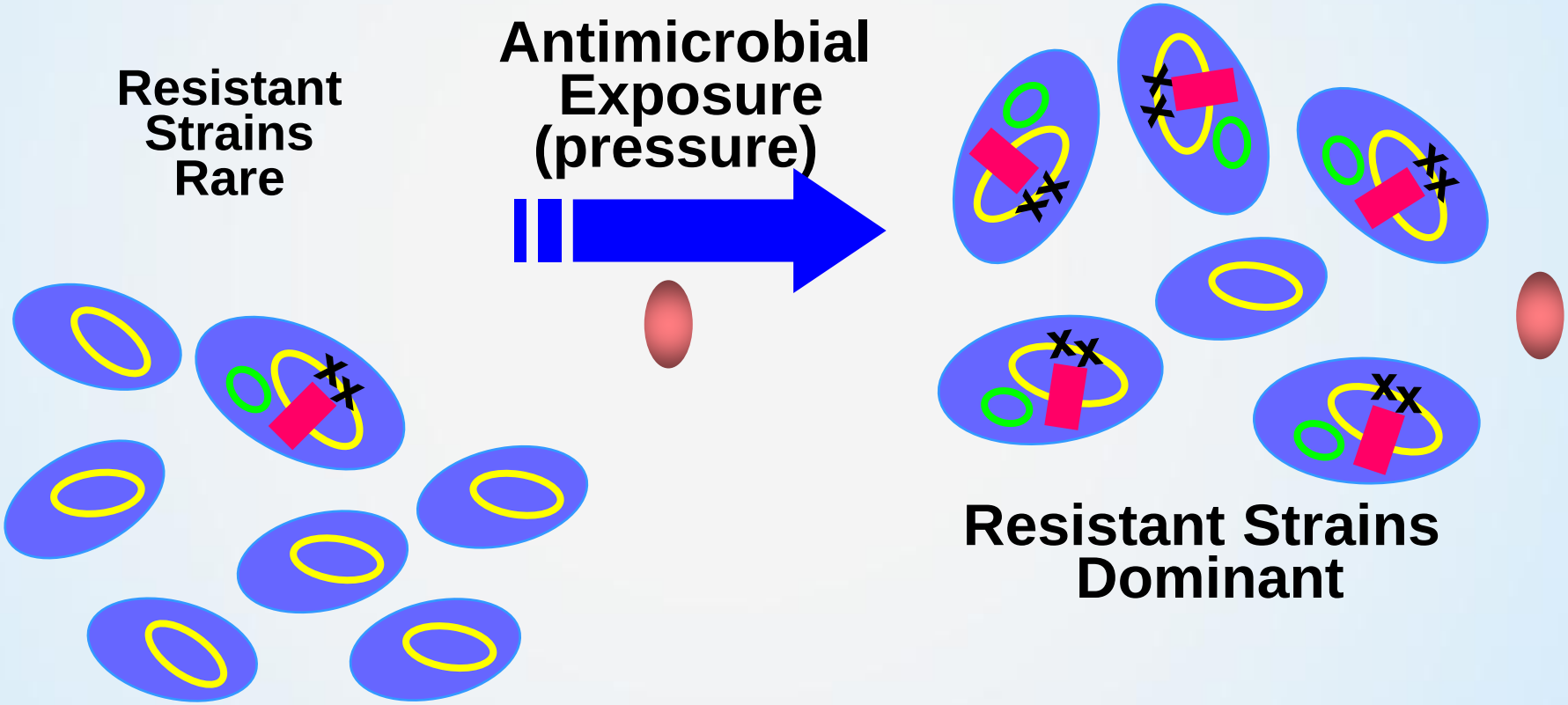


Chromosomal Resistance (Drug Resistant Mutants)	Plasmid Resistance (R Factor) and transposons
Spontaneous mutation → Altered target.	Presence of R factor (Plasmid that carry the genes of resistance).
Need the presence of antibiotic → selective pressure factor.	Not need for selective pressure factor.
Low frequency of transmission.	High frequency of transmission.
Less common.	More common.
e.g. mutation in P12 of 30S ribosomal subunit → Streptomycin resistance.	e.g. RP1 plasmid that encodes resistance to ampicillin.

Emergence of Antimicrobial Resistance



Selection for antimicrobial-resistant Strains



Genetic exchange of extra-chromosomal elements:

Plasmids:

- Bacteria often contain extra-chromosomal genetic elements called plasmids (R factors), are class of plasmids that carry genes for resistance to one or more antimicrobial drugs.
- Plasmids frequently carry genes that code for production of enzymes that inactivate or destroy antimicrobial agents (e.g. b-lactamase, which is effective against penicillins and cephalosporins and acetyl transferase, which destroys chloramphenicol).
- Plasmids may result in epidemic resistance bacteria by conjugation.
- Resistance may be transferred in the absence of the antimicrobial agent.

Transposons:

- These are short DNA sequences that carry and transfer many drug resistance genes.
- It occurs between one plasmid and another or between plasmid and a portion of the bacterial chromosome.

Evolution of Drug Resistance in *S. aureus*

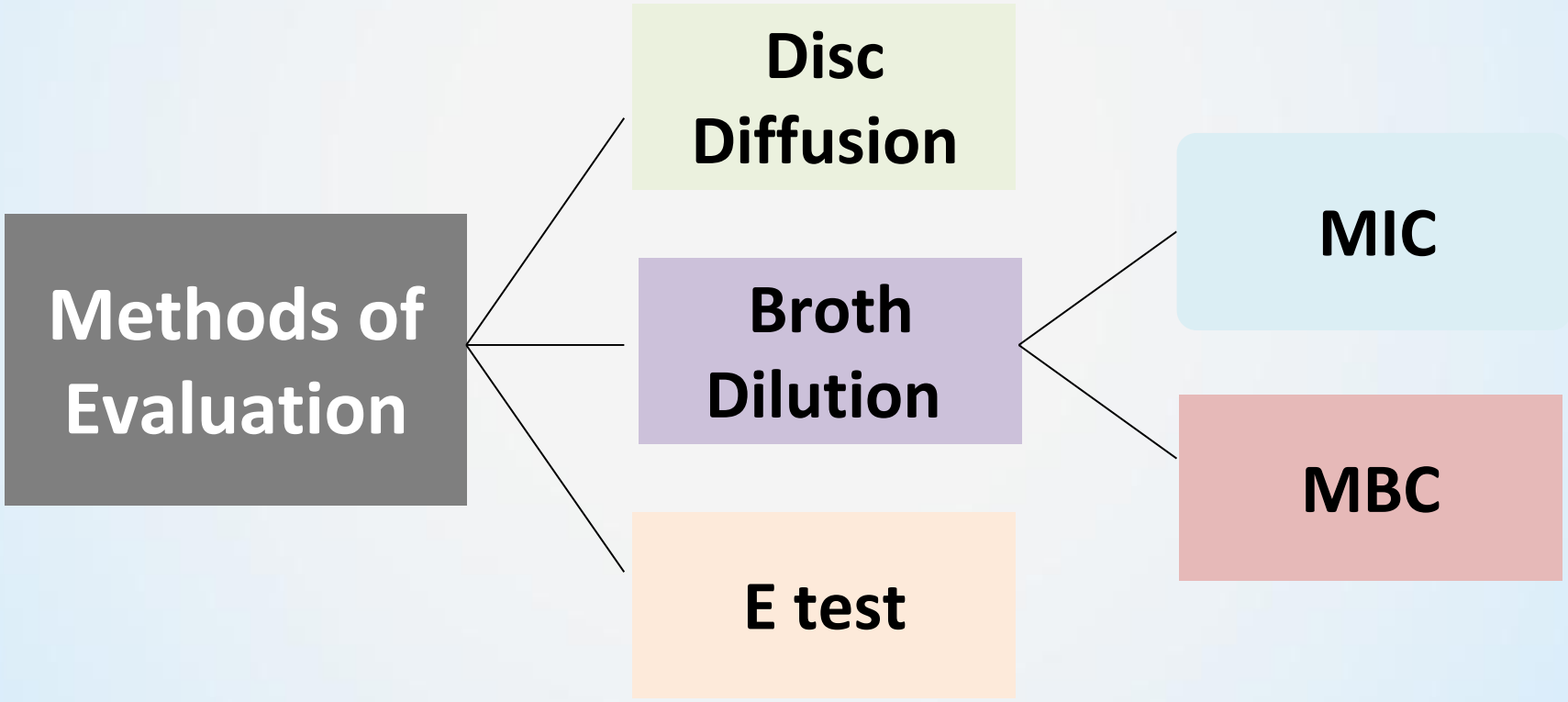
S. aureus → Penicillin → [1950s] → Penicillin-resistant *S. aureus* → Methicillin → [1970s] → Methicillin-resistant *S. aureus* (MRSA)

Methicillin-resistant *S. aureus* (MRSA) → Vancomycin → [1990s] → Vancomycin-resistant enterococci (VRE)

Methicillin-resistant *S. aureus* (MRSA) → [1997] → Vancomycin intermediate-resistant *S. aureus* (VISA)

VRSA

EVALUATION OF ANTIMICROBIAL ACTIVITY



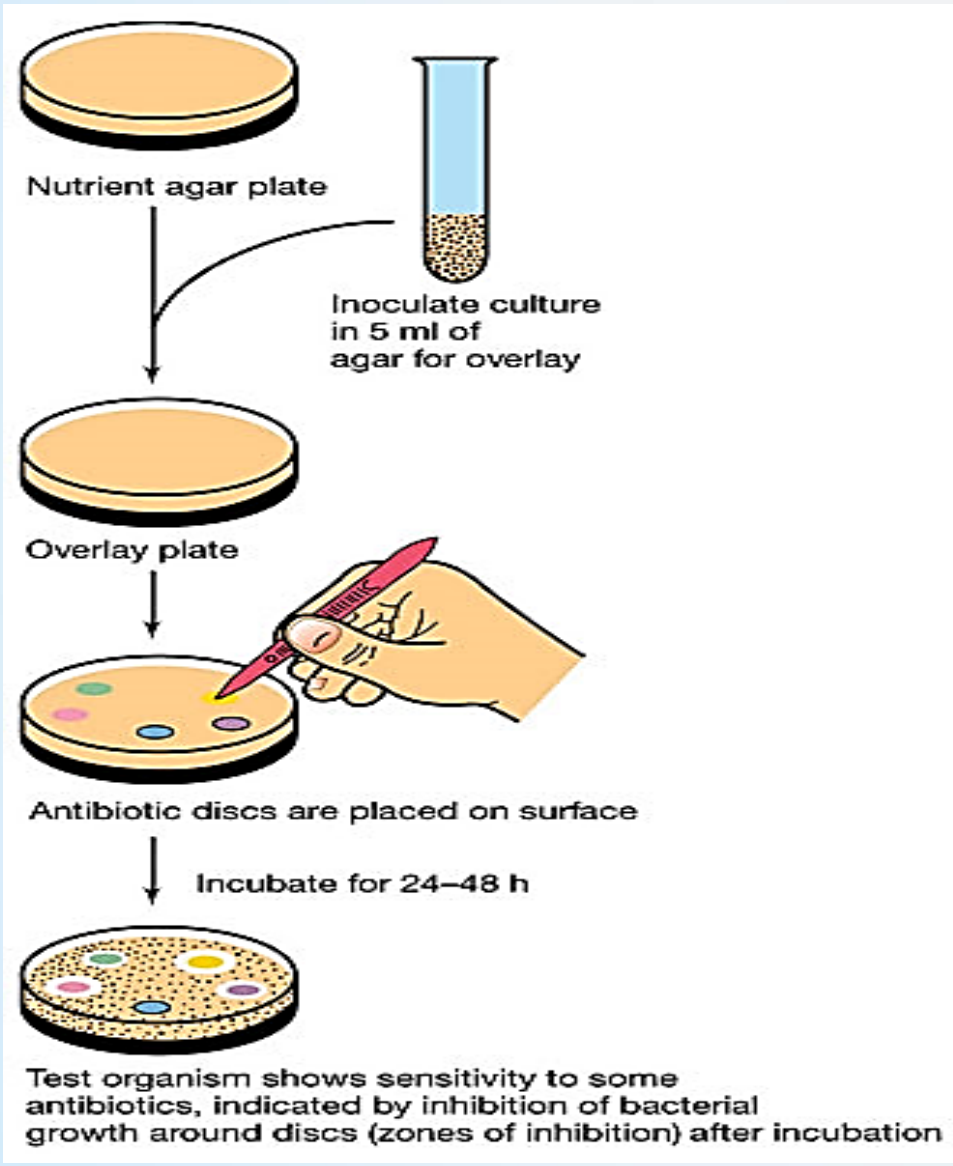
Disc Diffusion Method (Kirby - Bauer method):

Steps

- Filter papers discs are impregnated with a known amount of antimicrobial agents are placed on an agar surface (Muller - Hinton agar) that has been seeded with the organism to be tested.

- After 24 hours incubation, zones of inhibition of growth around the discs are measured and compared to a table of value that indicate susceptibility, intermediate susceptibility and resistance to each agent.

Disc Diffusion Method



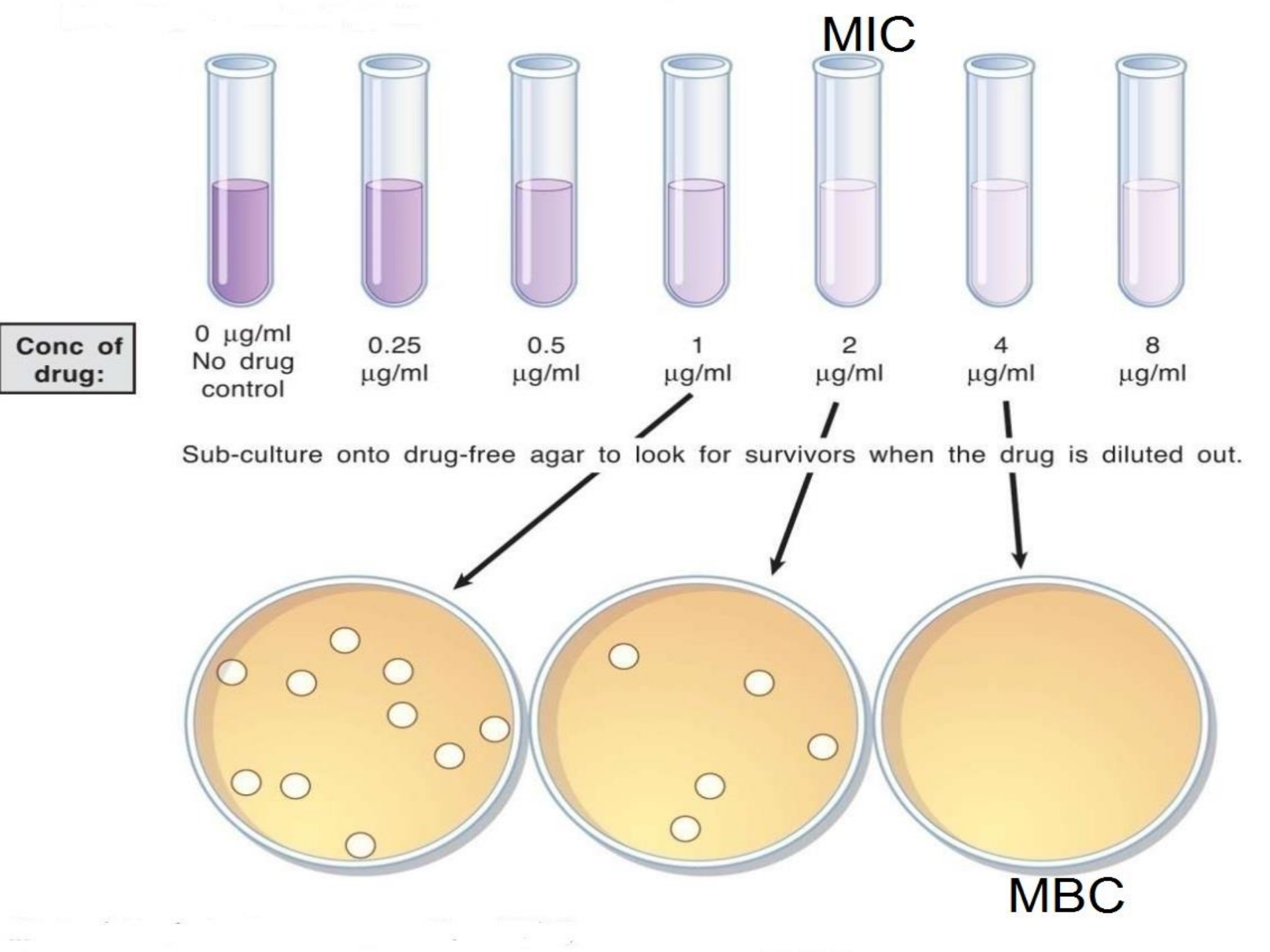
Dilution method:

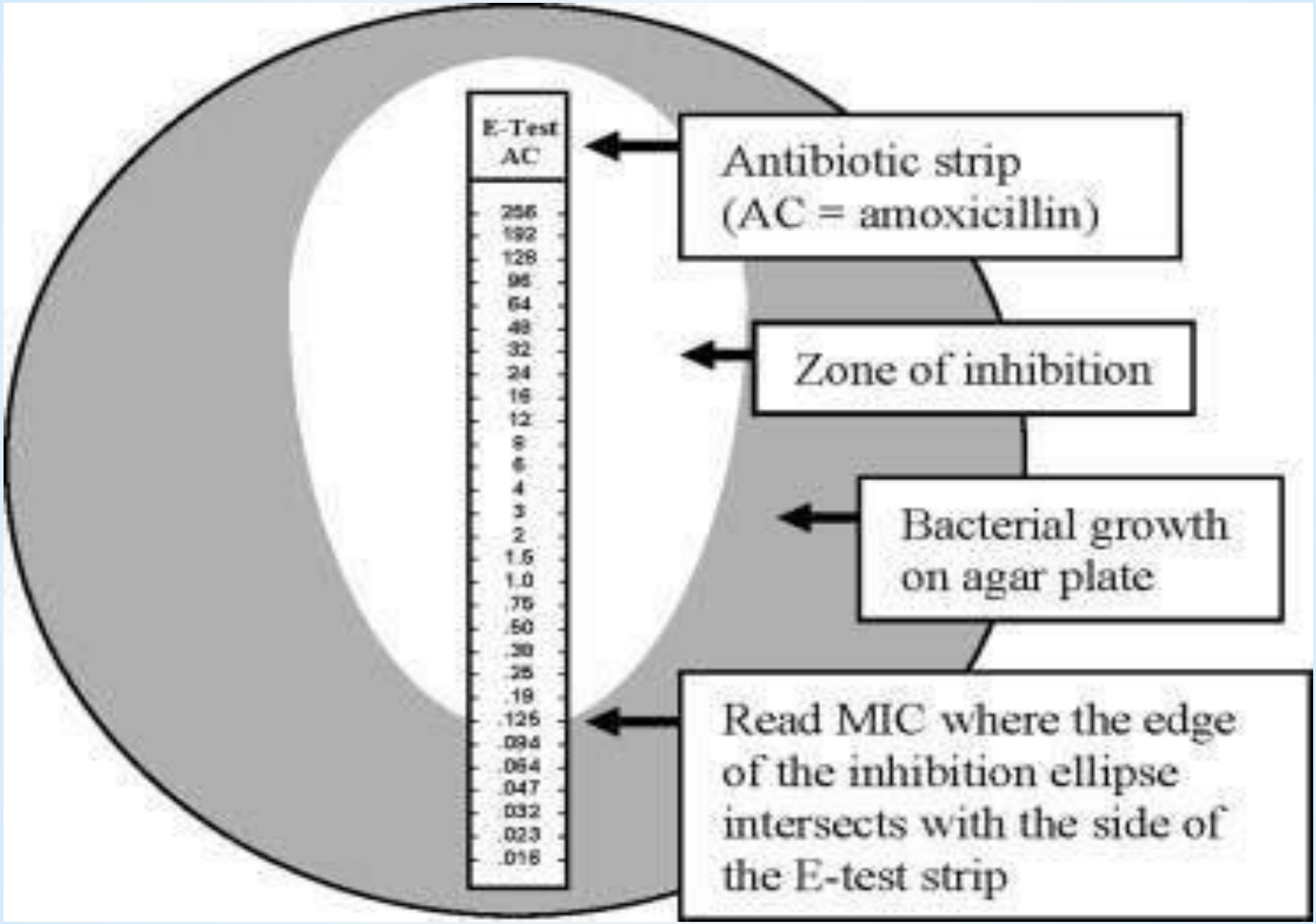
- To determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of an antimicrobial agent for a bacterial isolate.

- Graded amounts of antimicrobial agents are incorporated into liquid media.

- The media are subsequently inoculated with the bacteria and incubated.
- The end point (MIC) is taken, as the amount of antimicrobial substance required to inhibit the growth or to kill the test organism.

- MBC is determined by inoculating the drug free medium from the tube showed the MIC and after incubation, growth may be detected on the medium. So, MBC may be equal to or greater than the MIC.


MIC
Conc of drug:
0 µg/ml No drug control
0.25 µg/ml
0.5 µg/ml
1 µg/ml
2 µg/ml
4 µg/ml
8 µg/ml
Sub-culture onto drug-free agar to look for survivors when the drug is diluted out.
MBC


E-Test
AC
256
192
128
96
64
48
32
24
16
12
8
6
4
3
2
1.5
1.0
.75
.50
.38
.25
.19
.125
.094
.064
.047
.032
.023
.016
Antibiotic strip
(AC = amoxicillin)
Zone of inhibition
Bacterial growth
on agar plate
Read MIC where the edge
of the inhibition ellipse
intersects with the side of
the E-test strip

Case story or clinical correlate

- After laboratory antibiotic evaluation the case will receive the correct antibiotic with the correct dose.

Summary and rap up

- Antibacterial agents act by inhibition of cell wall synthesis, inhibition of cell membrane permeability, inhibition of protein or nucleic acid synthesis
- Resistance to antibiotics could be due to decrease intracellular accumulation, enzymatic inactivation or alteration of the target site.
- Evaluation of antimicrobial activity can be done by disc diffusion method, dilution method or E test.

Discussion 10 minutes

Brain storming question preferred as case senior

26 years female complaining from UTI when seeking medical care for the first time, her doctor prescribe an antibiotic with a specified dose, but signs and symptoms doesn't subside and when seeking medical advice again the case become complicated by severe pyelonephritis and urine retention.

- **What are the possible reasons for the failure of the initial antibiotic therapy in this patient?**
- **What are the common mechanisms by which bacteria develop resistance to antibiotics?**

MCQ Questions

Which of the following antibiotics is effective against Gram-negative bacteria by inhibiting DNA gyrase?

A. Penicillin
B. Amoxicillin
C. Ciprofloxacin
D. Chloramphenicol
E. Erythromycin

MCQ Questions

What is the most common mechanism of resistance to β-lactam antibiotics in bacteria?

A. Efflux pump activation
B. Alteration of ribosomal subunits
C. Production of β-lactamase enzymes
D. Mutation in DNA gyrase
E. Increased cell membrane permeability

References

- Department book
- Jawetz, Melnick, Adelberg's Medical Microbiology
- Color atlas and textbook of diagnostic microbiology Lippincott