Antibiotics

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Dr. Sulaiman Mahmoud Bani Abdel-Rahman

Bachelor degree in Medicine and Surgery - Mutah university

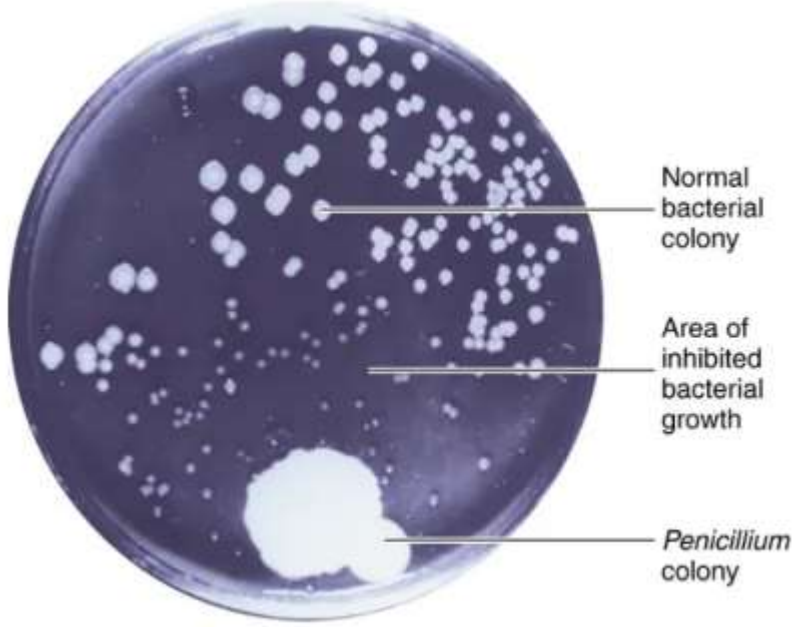
MSC Medical Microbiology – University of Manchester

PhD Medical Virology - University of Manchester

Discovery of Antimicrobial Agents

- In 1928, Alexander Fleming observed that the growth of the bacterium *Staphylococcus aureus* was inhibited in the area surrounding the colony of a mold that had contaminated a Petri plate

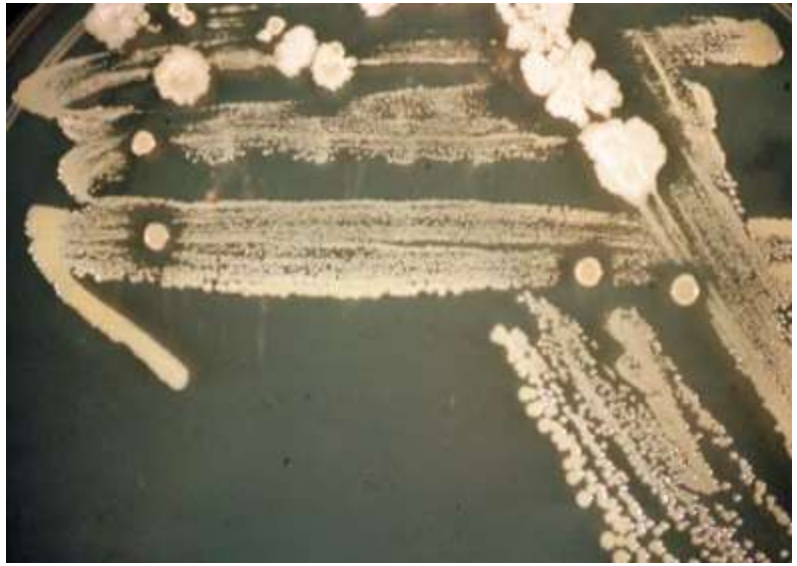
- The mold was identified as *Penicillium notatum*, and its active compound, which was isolated a short time later, was named penicillin.



Alexander Fleming took this photograph in 1928. The colony of *Penicillium* mold accidentally contaminated the plate and inhibited nearby bacterial growth.

Discovery of Antimicrobial Agents (cont)

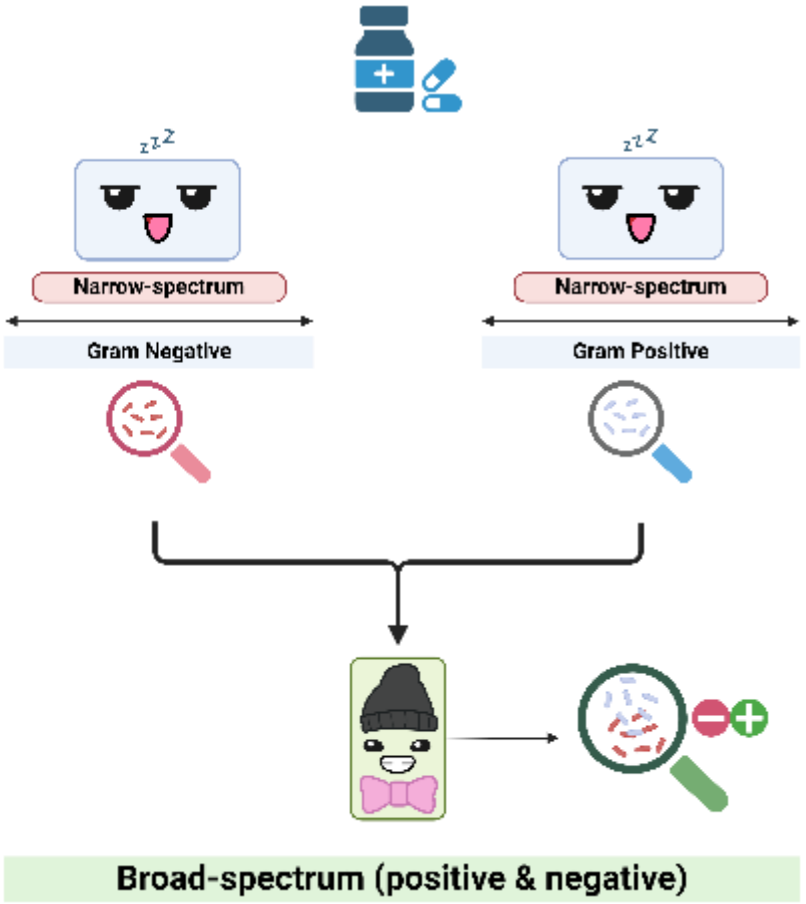
- Reactions between colonies on solid media are commonly observed in microbiology, and the mechanism of inhibition is called antibiosis
  - From this word comes the term **antibiotic**, a substance produced by microorganisms that in small amounts inhibits another microorganism.
- Therefore, the wholly synthetic sulfa drugs, for example, are technically antimicrobial drugs, not antibiotics, a distinction often ignored in practice.



Spectrum of Antimicrobial Activity

- **Narrow-spectrum:** the drugs that only act on Gram-positive **OR** Gram-negative bacteria.
  - Positive: such as Penicillin, and Vancomycin

- **Broad-spectrum:** the drugs that have act on Gram-positive **AND** Gram-negative bacteria.



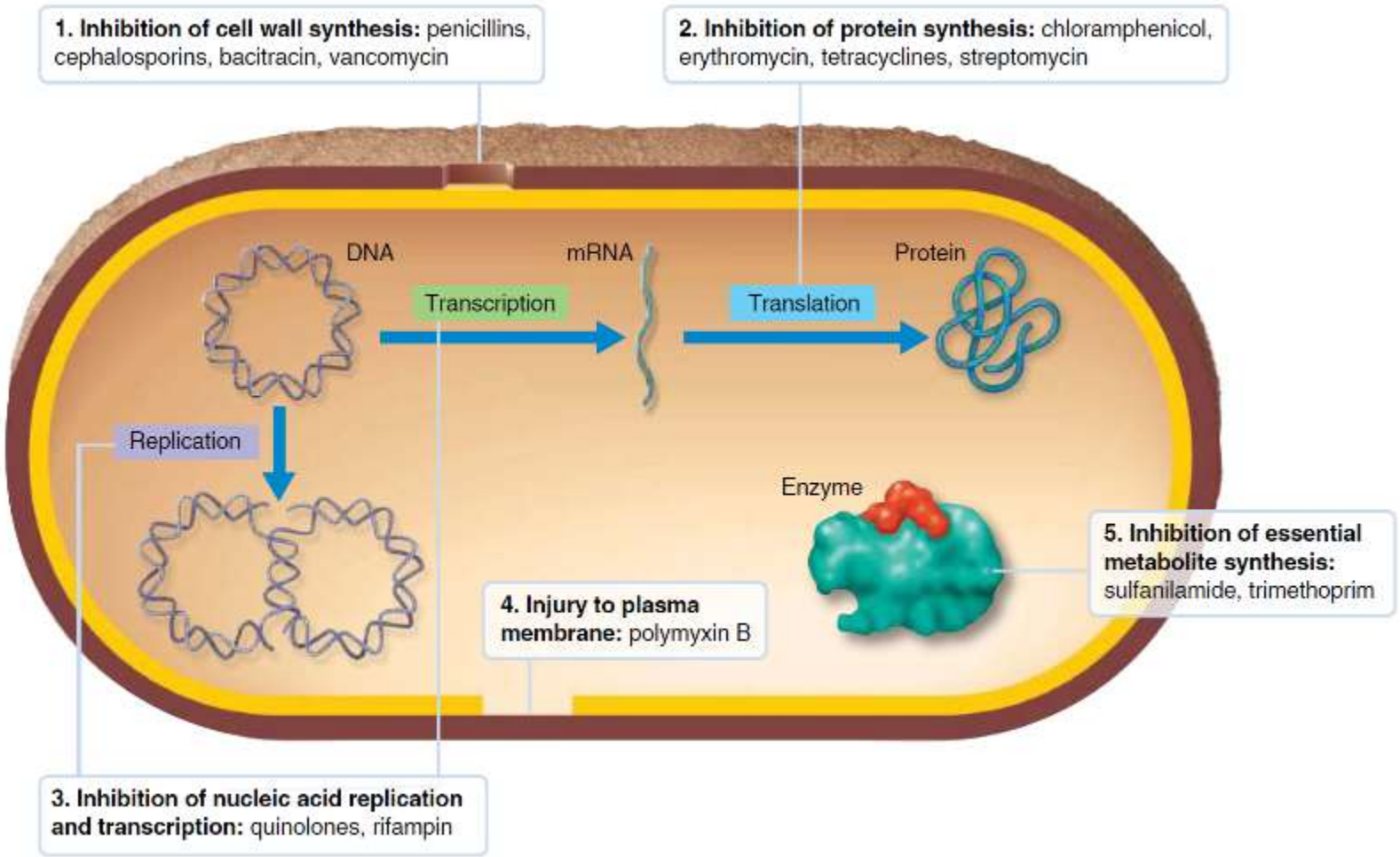
The Action of Antimicrobial Drugs

- Antimicrobial drugs are either
  - **Bactericidal** → they kill microbes directly

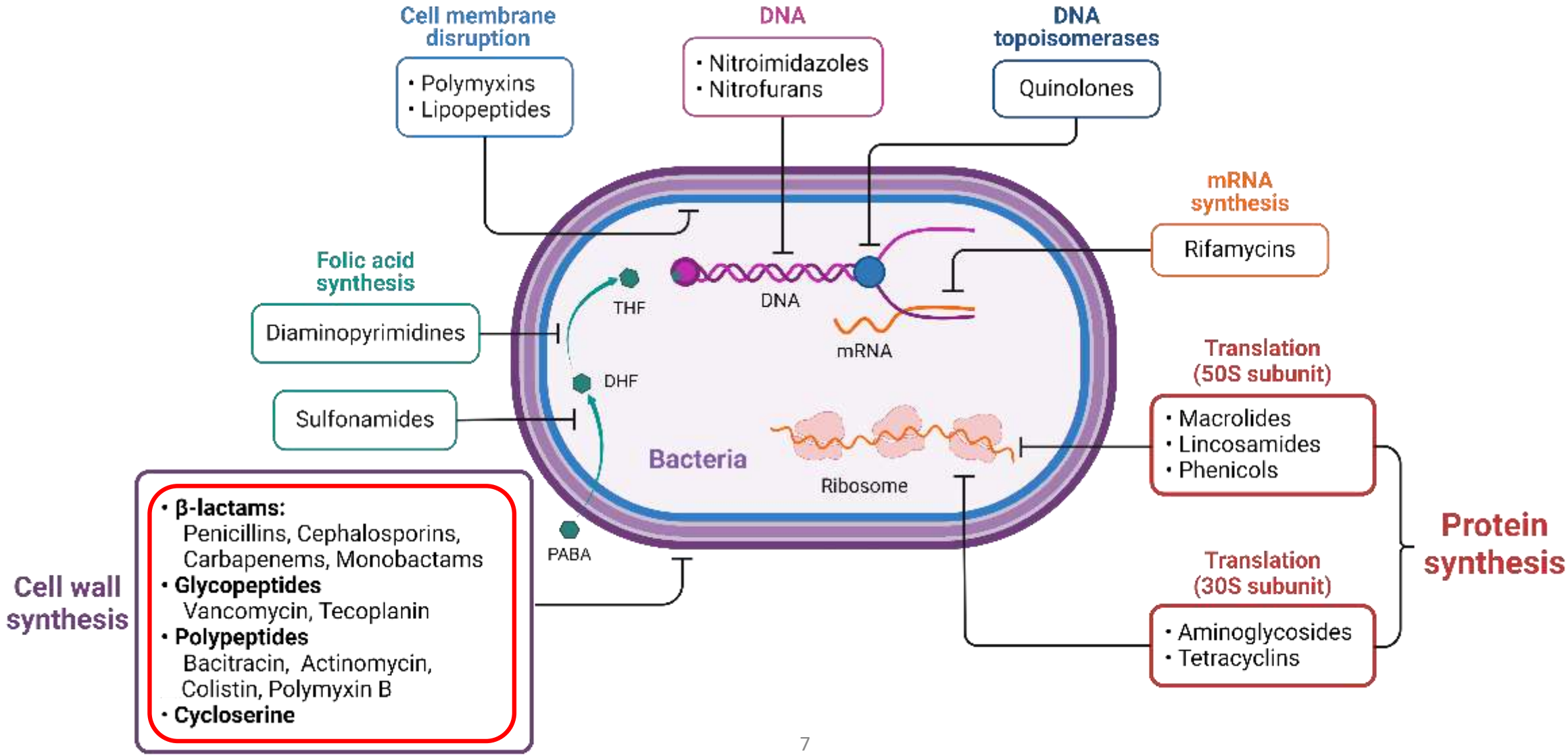
OR

  - **Bacteriostatic** → they prevent microbes from growing
    - the host's own defenses, such as phagocytosis and antibody production, usually destroy the microorganisms

Antimicrobial Therapy Targets

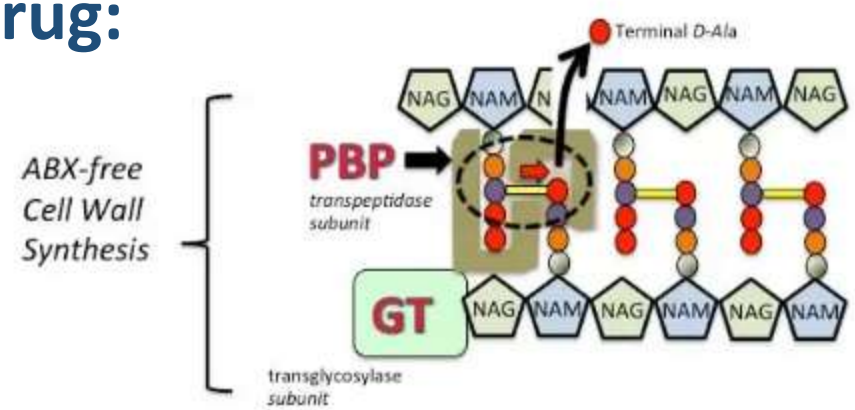


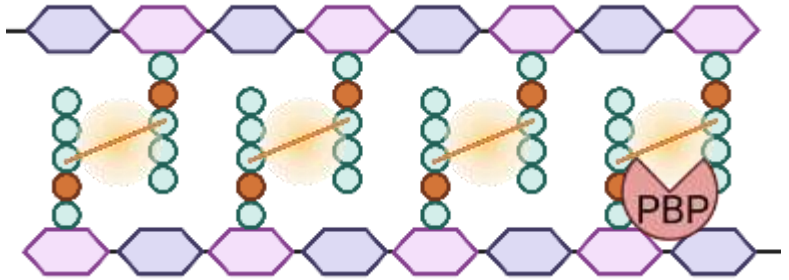
Antimicrobial Therapy Targets



Inhibition of cell wall synthesis

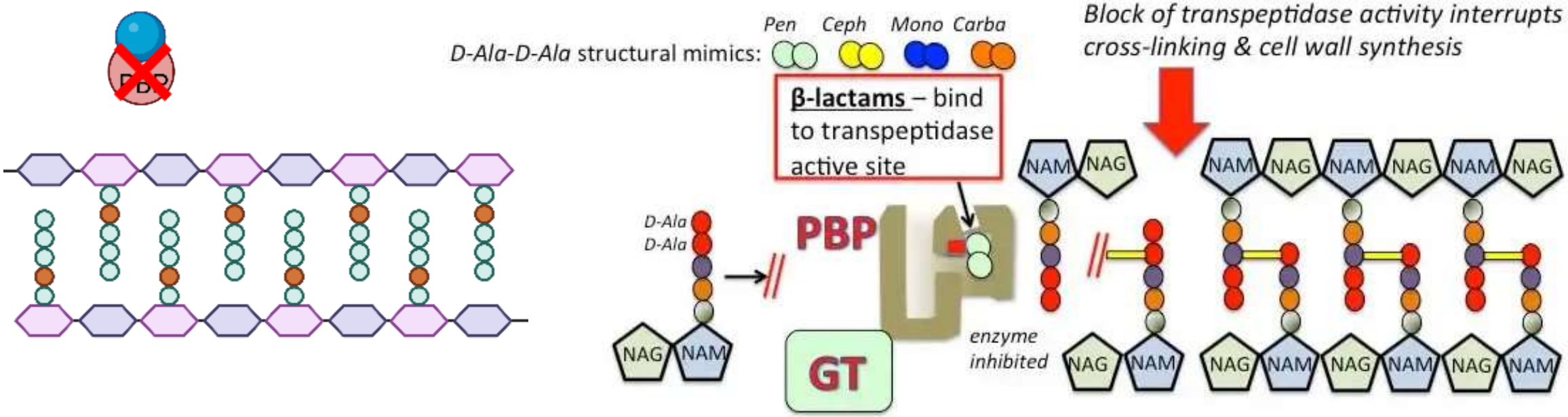
1. **In absence of drug:**





Inhibition of cell wall synthesis

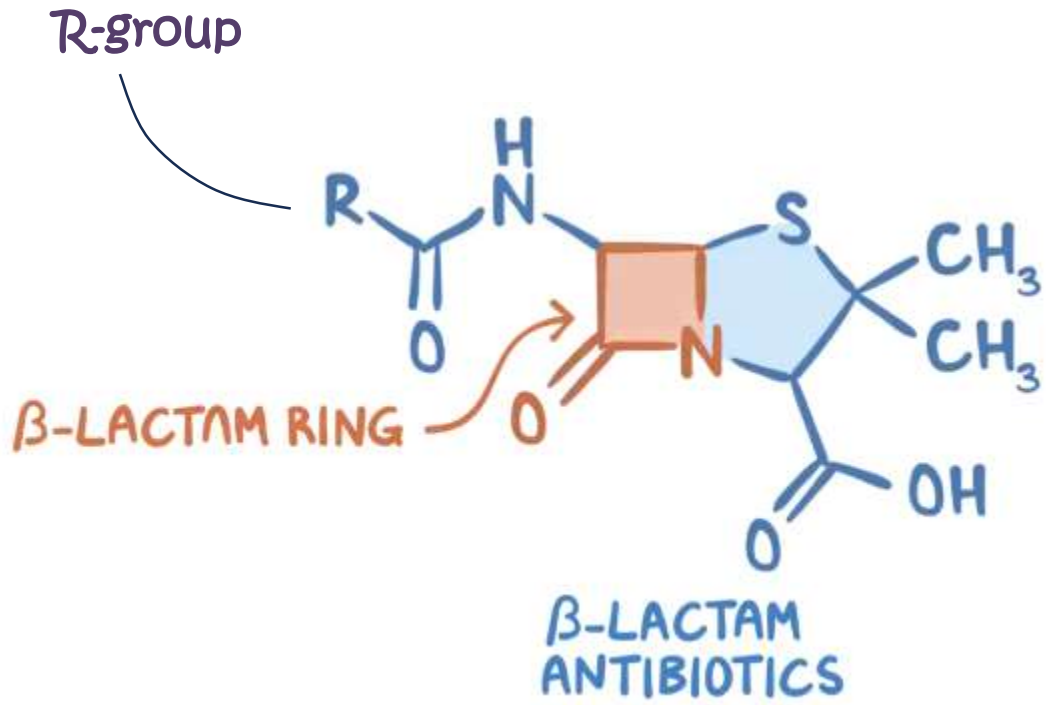
2. In the presence of the drug (beta-lactams):



Beta lactam interaction with penicillin binding proteins (PBP) blocks cross linking and compromises cell wall rigidity.

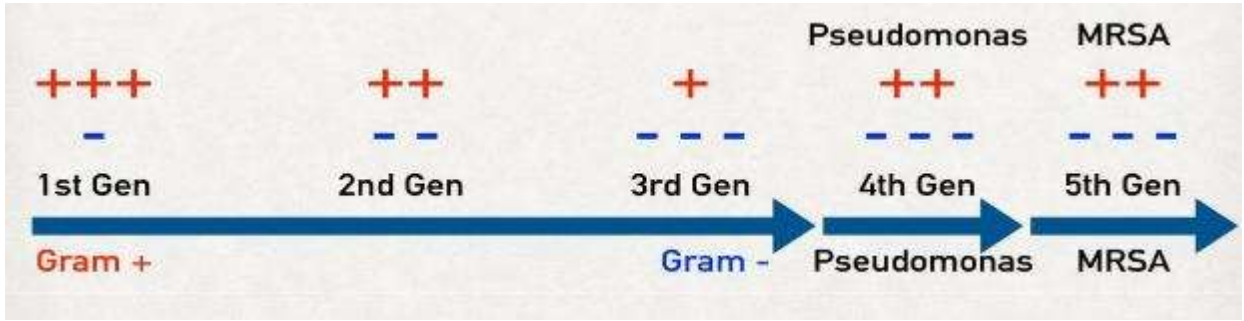
Beta-lactamase destroys beta-lactam ring ☹

- Beta-lactamase Enzymes destroy the Beta-lactam Ring
  - R-group → changes the structure of the antibiotic itself
  - Clavulanic Acid → inhibits beta-lactamase enzymes produced by resistant bacteria → resembles beta-lactam antibiotics structurally and “tricks” beta-lactamase enzymes into binding with it.
- R group functions in beta-lactam antibiotics:
  - Modifying Spectrum of Activity
  - Enhancing Stability against Beta-lactamase Enzymes → changes the structure of the antibiotic itself → beta-lactamase enzyme can not bind to the antibiotic



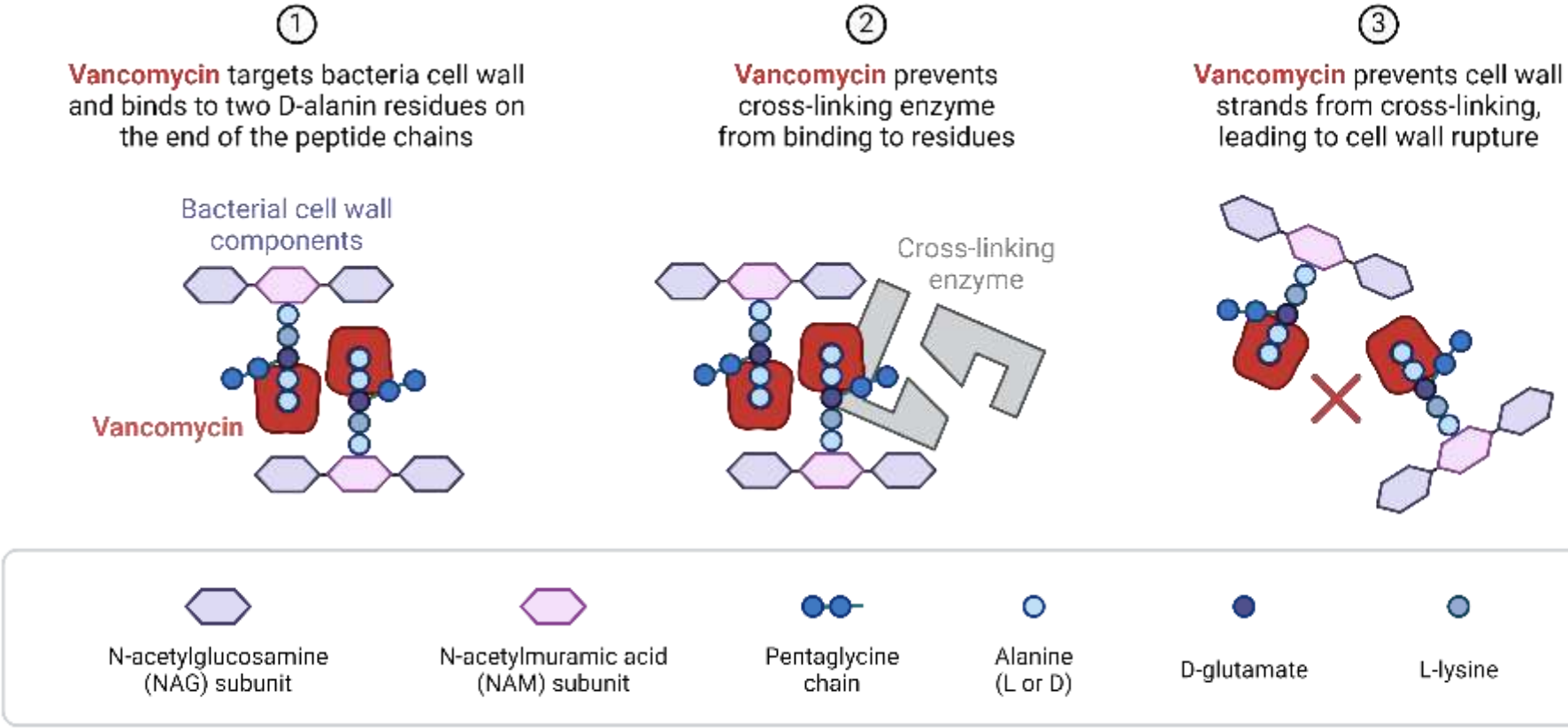
Cephalosporins Gram coverage

Gram coverage



Inhibition of cell wall synthesis

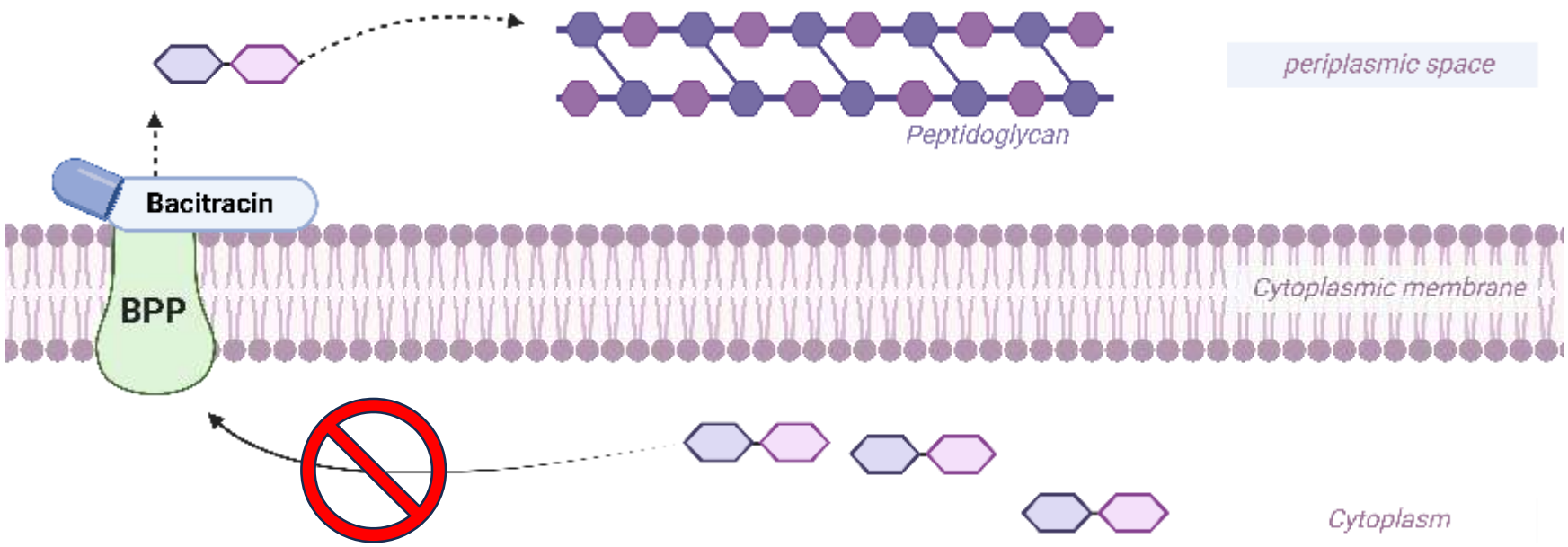
3. In the presence of the drug (Glycopeptides):



Glycopeptides bind to (they do not mimic) D-Ala-D-Ala termini leading to inhibition of transpeptidases and transglycosylases.

Inhibition of cell wall synthesis

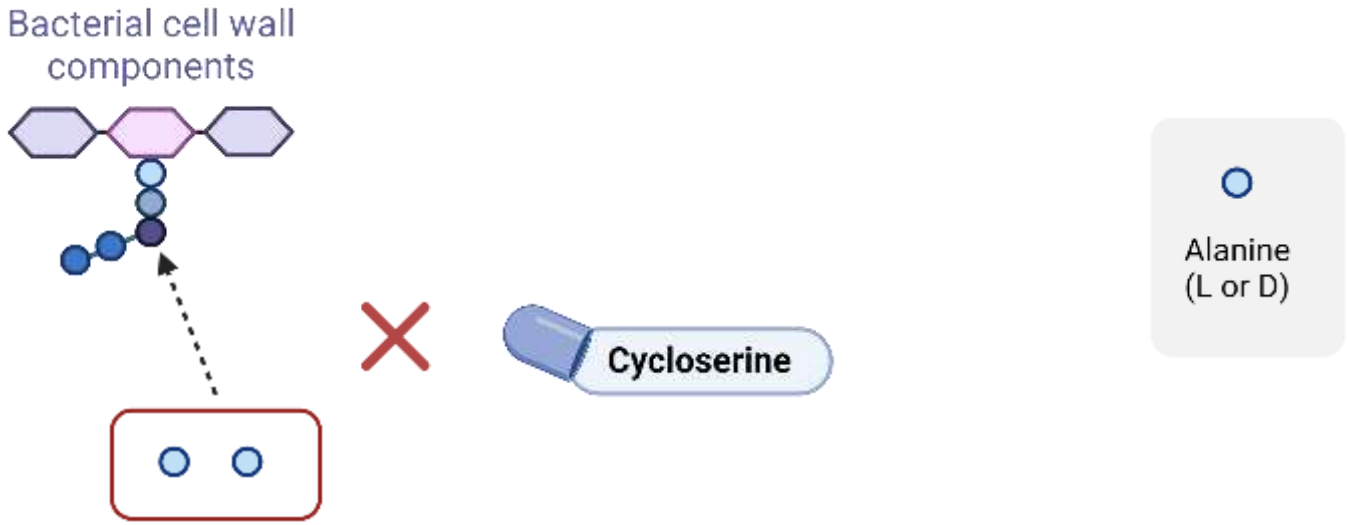
4. In the presence of the drug (Bacitracin):



By binding to bactoprenol, Bacitracin prevents the dephosphorylation of this lipid carrier, effectively halting the transport of peptidoglycan precursors (NAG & NAM)

Inhibition of cell wall synthesis

5. In the presence of the drug (Cycloserine:):



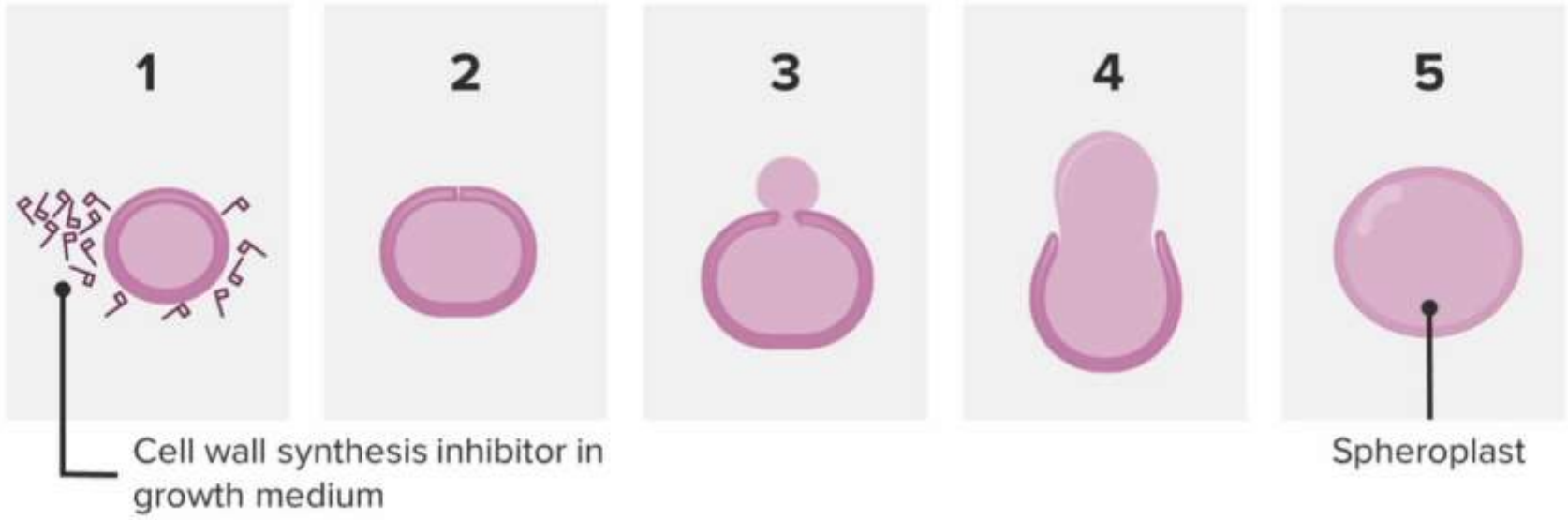
Cycloserine is a structural analog of D-alanine, an amino acid essential for peptidoglycan synthesis.
It competitively inhibits two enzymes: alanine racemase and D-alanine ligase.

- Alanine racemase converts L-alanine to D-alanine, a necessary step for the formation of the D-Ala-D-Ala dipeptide.
- By inhibiting D-alanineligase, cycloserine blocks the formation of the D-Ala-D-Ala link in peptidoglycan precursors, stopping cell wall synthesis.

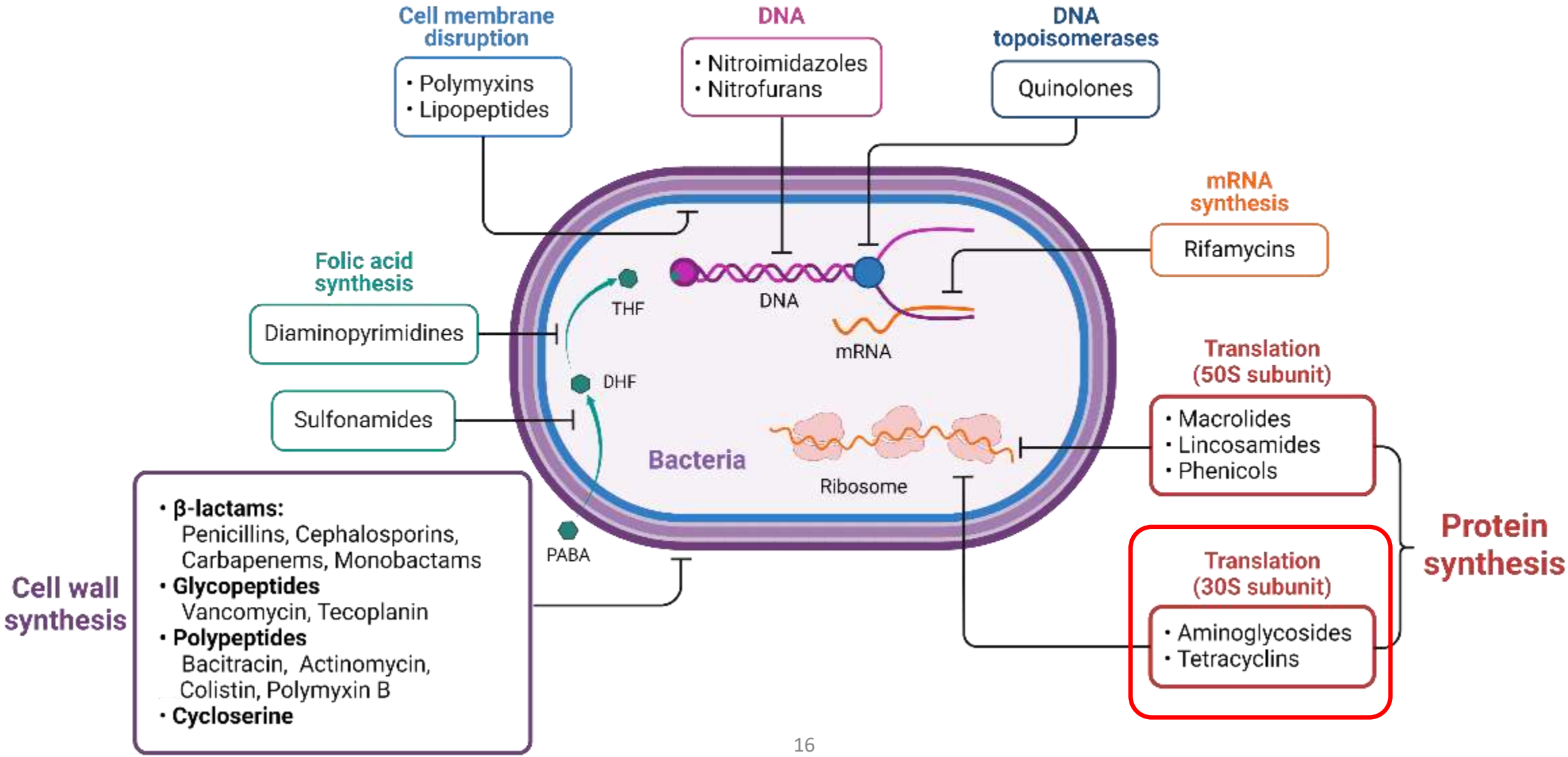
Inhibition of cell wall synthesis

So, no cell wall synthesis → what next?

The dividing cell can not build new cell wall → **Spheroplasts** will autocatalyze and die



Antimicrobial Therapy Targets



Inhibition of protein synthesis (30S)

Antimicrobials that Bind to the 30S Ribosomal Subunit

Aminoglycosides

Streptomycin
Kanamycin
Gentamicin
Tobramycin
Amikacin
neomycin (topical)

Tetracyclines

Minocycline
doxycycline

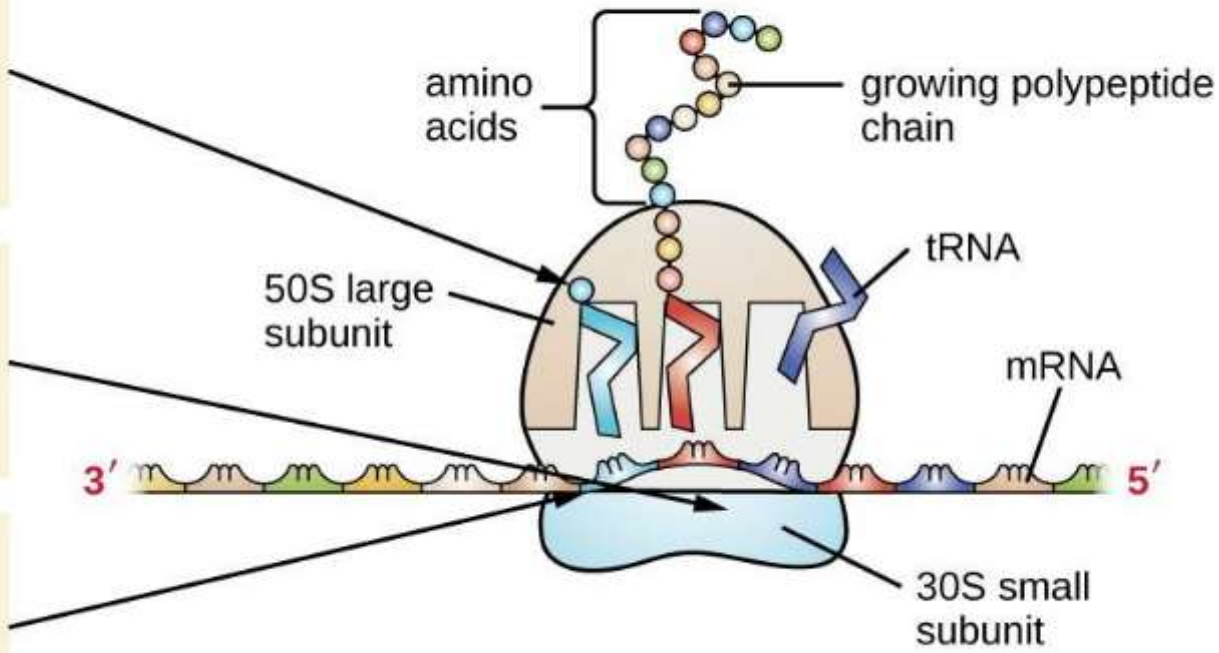
Aminoglycosides

Major classes of protein synthesis–inhibiting antibacterials

Chloramphenicol, macrolides, and lincosamides
- Bind to the 50S ribosomal subunit
- Prevent peptide bond formation
- Stop protein synthesis

Aminoglycosides
- Bind to the 30S ribosomal subunit
- Impair proofreading, resulting in production of faulty proteins

Tetracyclines
- Bind to the 30S ribosomal subunit
- Block the binding of tRNAs, thereby inhibiting protein synthesis

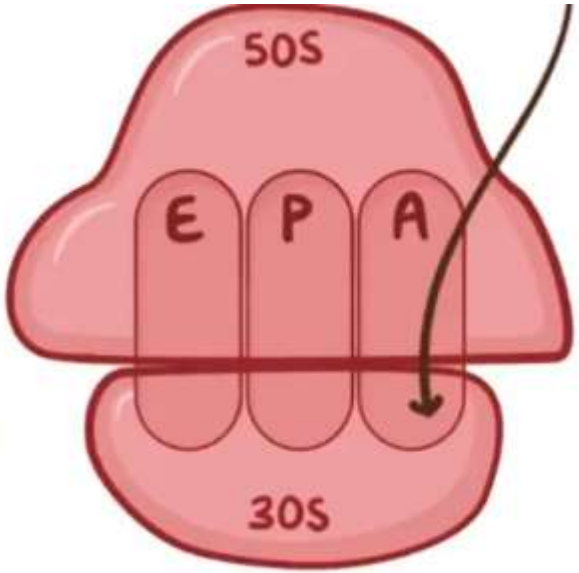


Inhibition of protein synthesis (30S) Aminoglycosides

- Irreversibly bind to the 30S subunits
  - Interfere with the proofreading process, thus causing errors in the protein's amino acid sequence
    - These faulty proteins will eventually lead to the death of the bacteria.
  - Prevent the formation of the ribosome-mRNA complex
    - reducing the amount of proteins being synthesized.
  - Inhibiting the initiation of protein synthesis
- Gram positive bacteria have a thicker cell wall compared to Gram negative bacteria, so aminoglycosides can't penetrate them.
- **Resistance** – Common

Inhibition of protein synthesis (30S)
Tetracyclines

* BIND to A-SITE on 30S
  - ↳ INHIBITS BINDING of tRNAs to mRNA-RIBOSOME COMPLEX
    - ↳ SHUTS DOWN PROTEIN SYNTHESIS

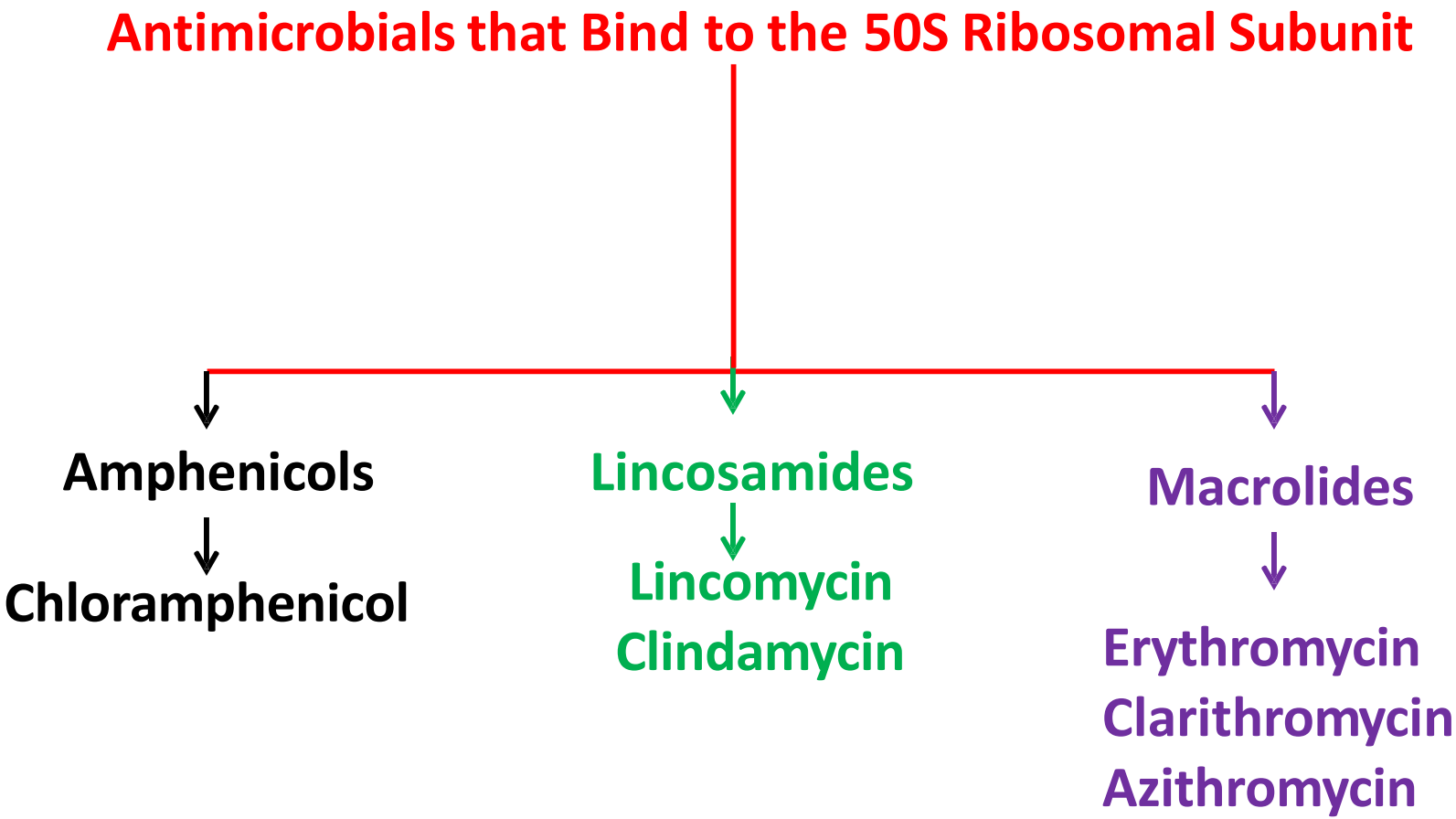


Mode of action - The tetracyclines reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA

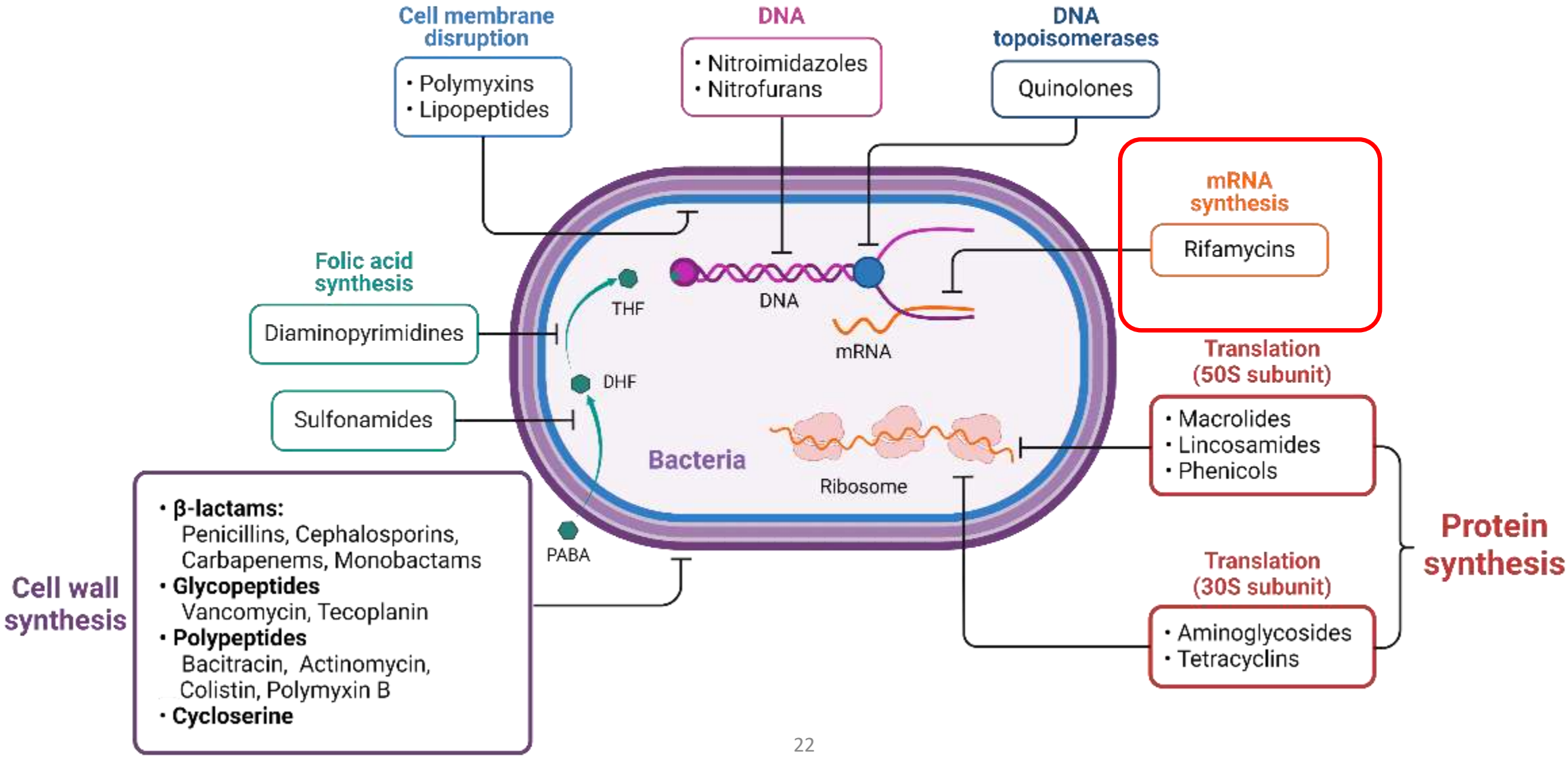
Spectrum of activity - Broad spectrum; Useful against intracellular bacteria

Resistance – Common

Inhibition of protein synthesis (50S)



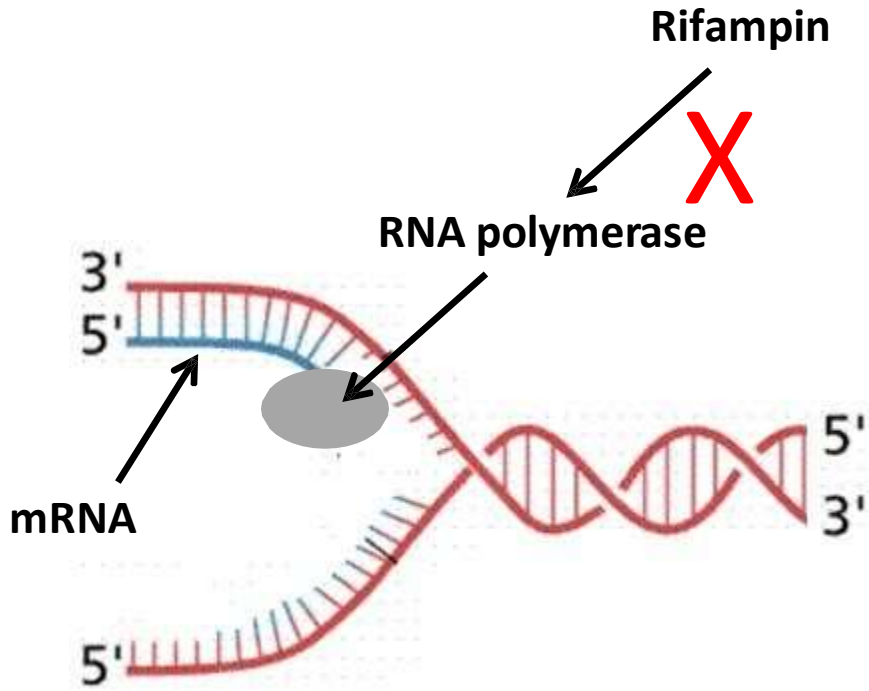
Antimicrobial Therapy Targets



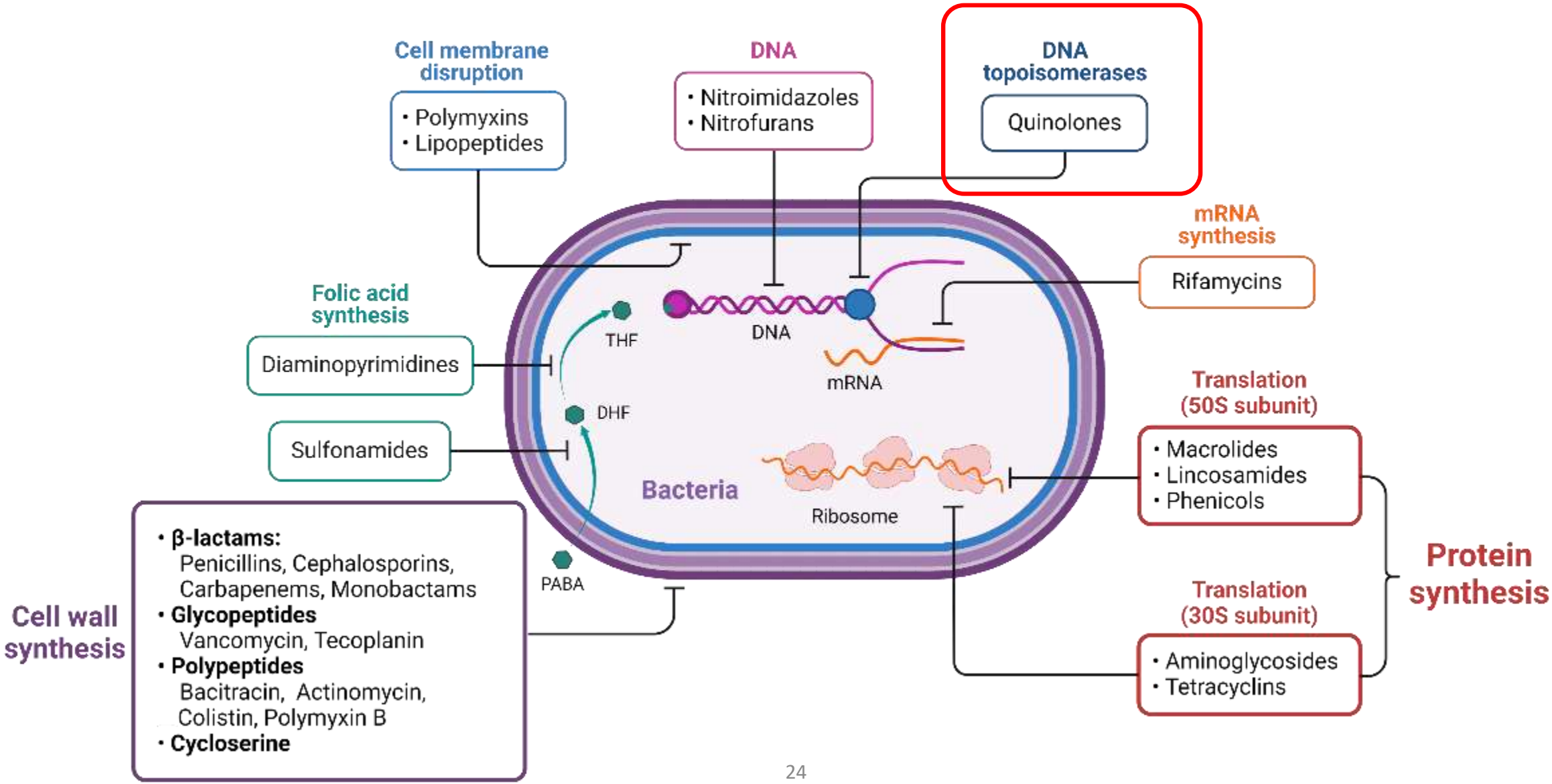
Inhibitors of mRNA Synthesis

Rifamycins group:
Rifampin, Rifampicin, Rifabutin

- **Selectivity due to** differences between prokaryotic and eukaryotic RNA polymerase
- **Mode of action:** these antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of mRNA synthesis.
- **Resistance**: Common



Antimicrobial Therapy Targets



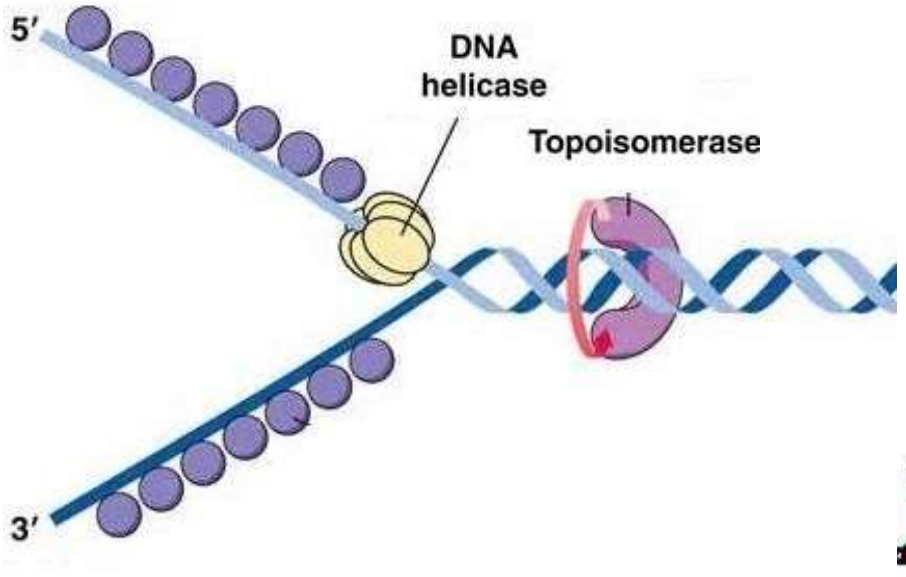
Inhibitors of DNA Synthesis

Fluoroquinolones:

nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin.

Mode of action - These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase II) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

Resistance - Common for nalidixic acid

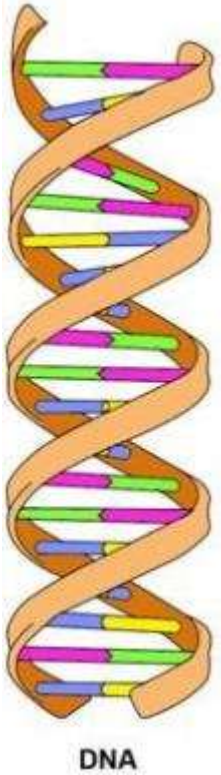


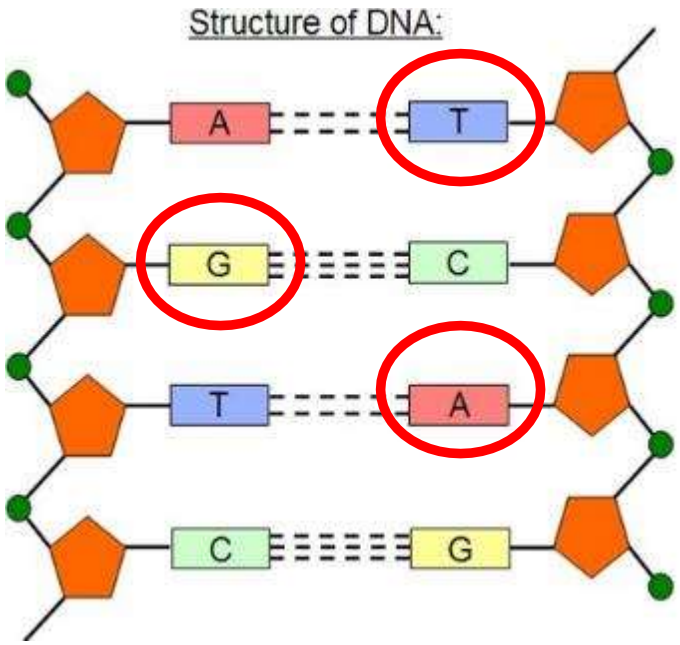
Supercoiling refers to the extra twisting or coiling of DNA that compacts it and helps fit the long DNA molecules within the limited space of a bacterial cell.

Inhibition of nucleic acid synthesis

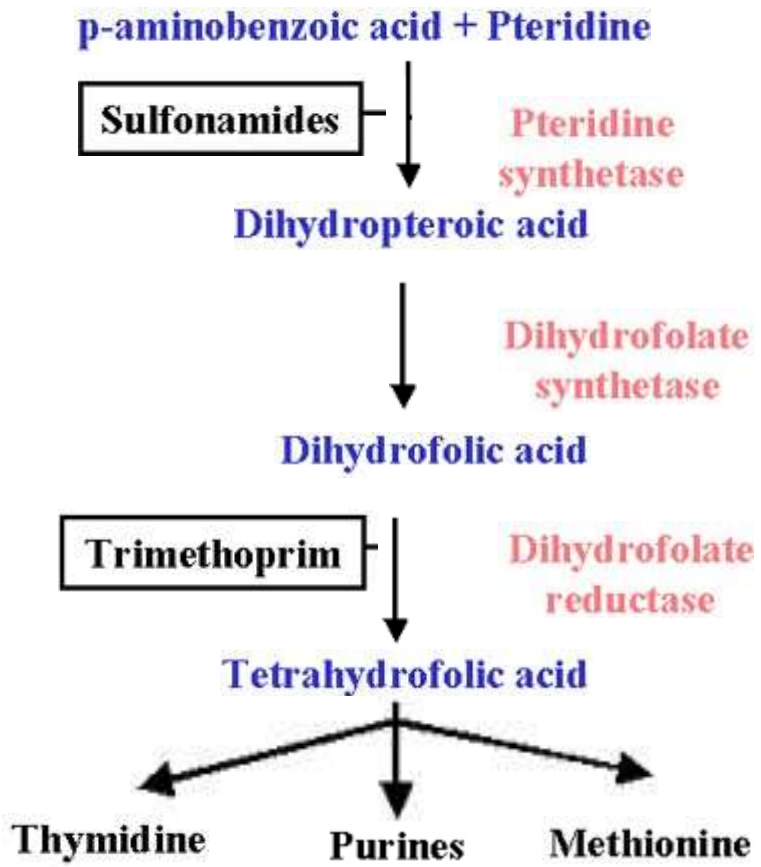
Nucleic acid synthesis is inhibited by:

1. **Trimethoprim**
2. **Sulfonamide group:** Sulfamethoxazole, Sulfadiazine Sulfathiazole, Sulfamerazine





Purine: adenine & guanine
Pyrimidine: cytosine & thymine



(Amino Acid)

Interference with cell membrane integrity

- Polymyxin B: binds to membrane of Gram negative bacteria and alters permeability
- This leads to leakage of cellular contents and cell death
- These drugs also bind to eukaryotic cells to some extent, which limits their use to topical applications

Antimicrobial Drug Resistance Principles and Definitions

- Antimicrobial resistance refers to development of resistance co an antimicrobial agent by a microorganism. It can be of two types:
  1. Acquired
  2. Intrinsic.

- Resistance provides a selective advantage

- Resistance can result from single or multiple steps

Intrinsic Resistance

- Intrinsic resistance is the innate ability of a bacterial species to resist the activity of a particular antimicrobial agent through inherent structural or functional characteristics, allowing tolerance of a particular drug or antimicrobial class.
- Such natural resistance can be caused by the following:
    1. inability of the drug to enter the bacterial cell (**Impermeability**)
    2. innate production of enzymes that inactive the drug (**Enzymatic Inactivation**).
    3. lack of affinity of the drug for the bacterial target

Example 1: The intrinsic resistance of gram-negative bacteria to vancomycin is an example of their outer membrane being impermeable to the large, rigid, and hydrophobic glycopeptide molecule vancomycin.

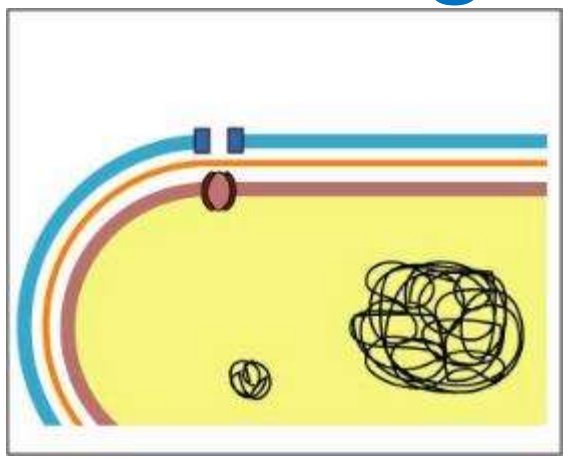
Example 2: β-Lactamases hydrolyze β-lactam antibiotics (*Enterobacter species* and *Pseudomonas aeruginosa*)

Acquired Mechanisms of Resistance

1. **Efflux:** Although efflux plays a major role in intrinsic resistance, changes in the cell wall proteins can also result in novel acquired traits
2. **Target Site Modification:** Mutations in these PBPs lead to alteration of PBPs, which result in reduced affinity for β-lactam antibiotics
3. **Enzymatic Inactivation:** Examples are enzymes that mediate hydrolysis of the β-lactam ring of β-lactam antibiotics (Staphylococcus aureus have acquired genes that produce beta-lactamase (e.g., via plasmids)

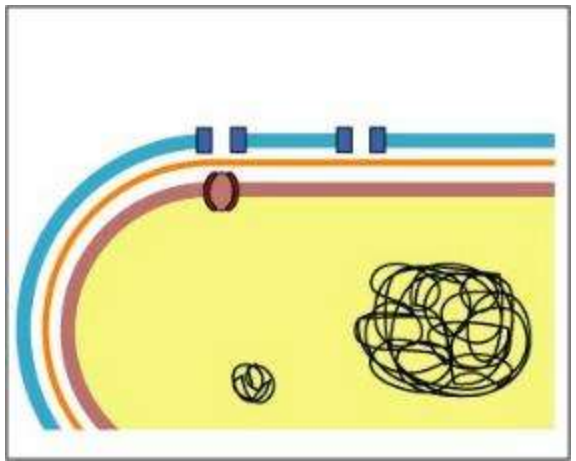
Principles of Antimicrobial Drug Resistance

- Altered permeability
  - Altered influx
    - Gram negative bacteria

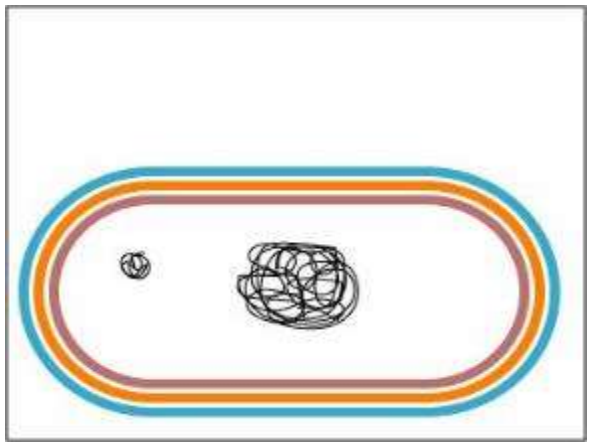


Altered permeability

- Altered efflux
  - tetracycline

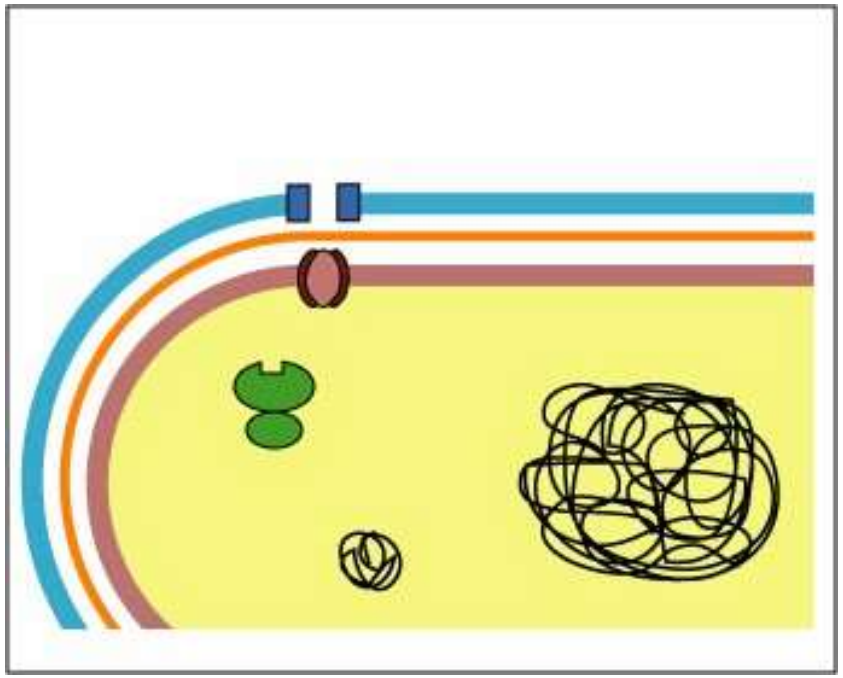


- Inactivation
  - Beta-lactamase



Antimicrobial Drug Resistance Principles and Definitions

- Altered target site
  - Penicillin binding proteins
  - RNA polymerase
  - 30S ribosome



Question

- Why beta-lactams are not effective against mycoplasma pneumonia?
- Resistance? Which type?