Antibiotics

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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.

Normal bacterial colony

Area of inhibited bacterial growth

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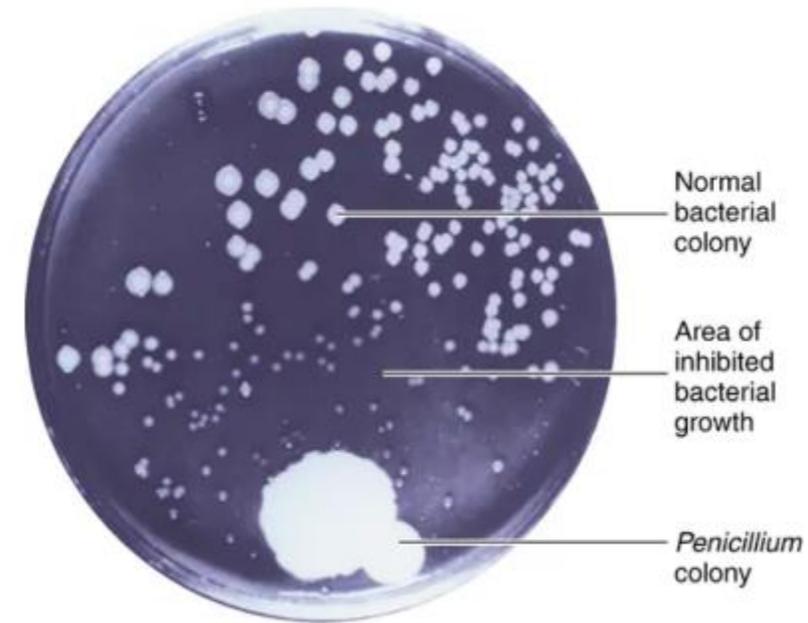
Alexander Fleming took this photograph in 1928. The colony of *Penicillium* mold accidentally contaminated the plate and inhibited nearby bacterial growth.

Image Zoom in

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.
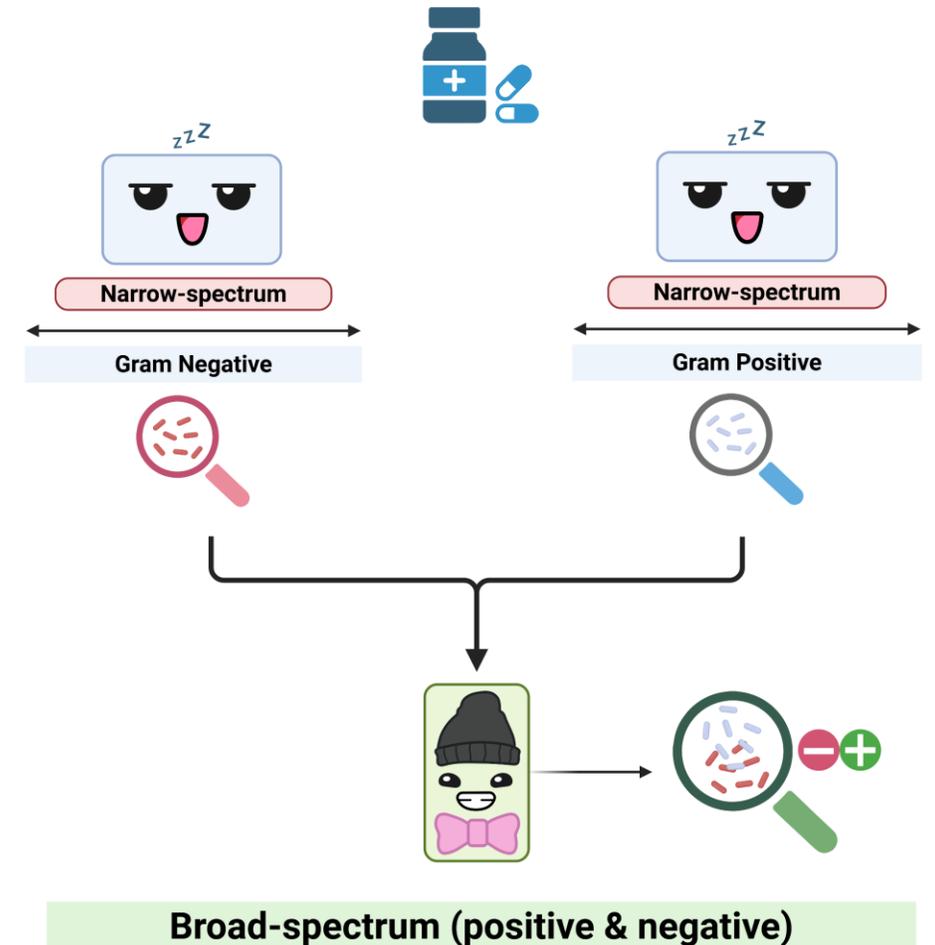
The Action of Antimicrobial Drugs

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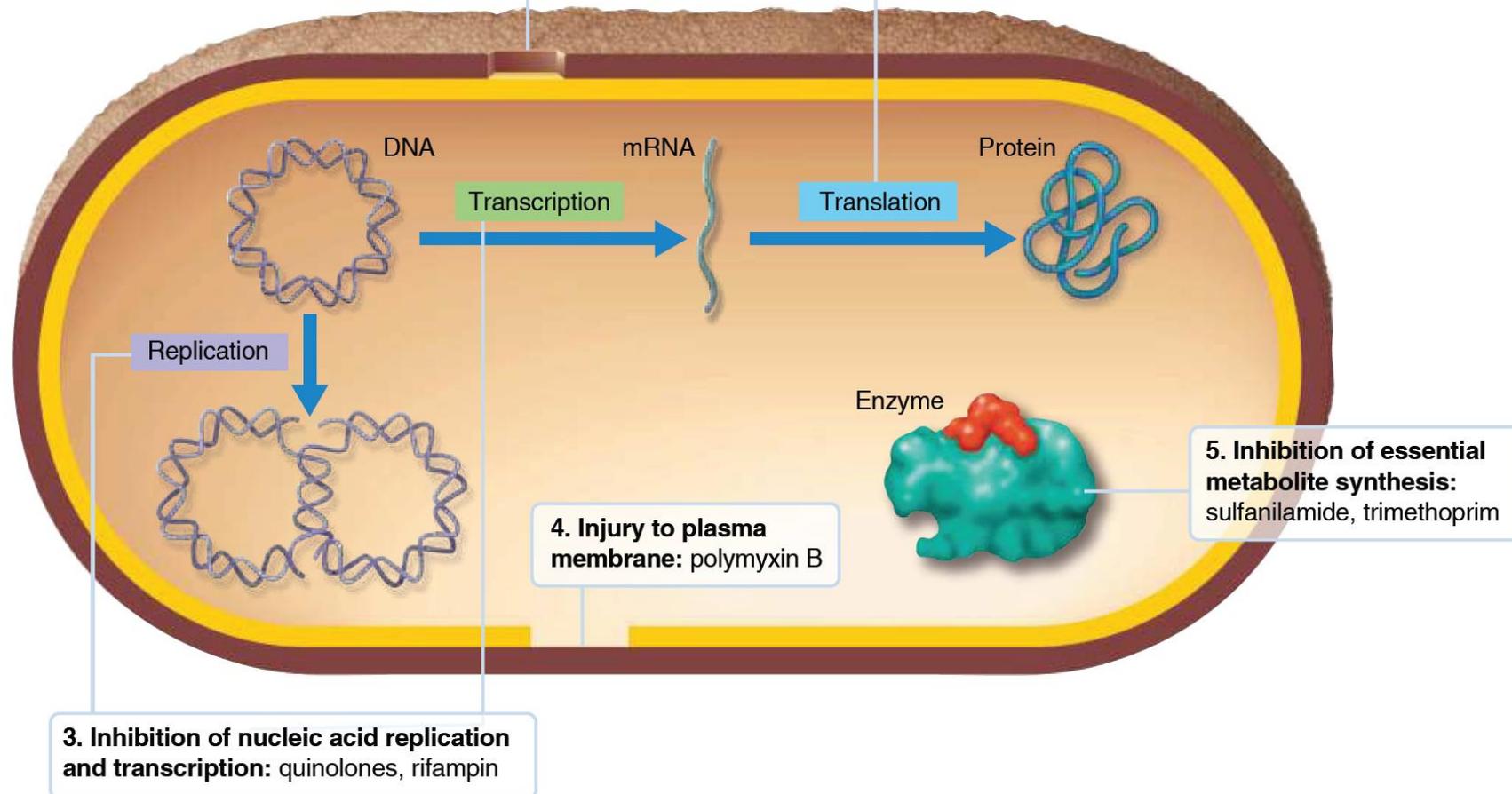 - **Bactericidal** → they kill microbes directly

  OR

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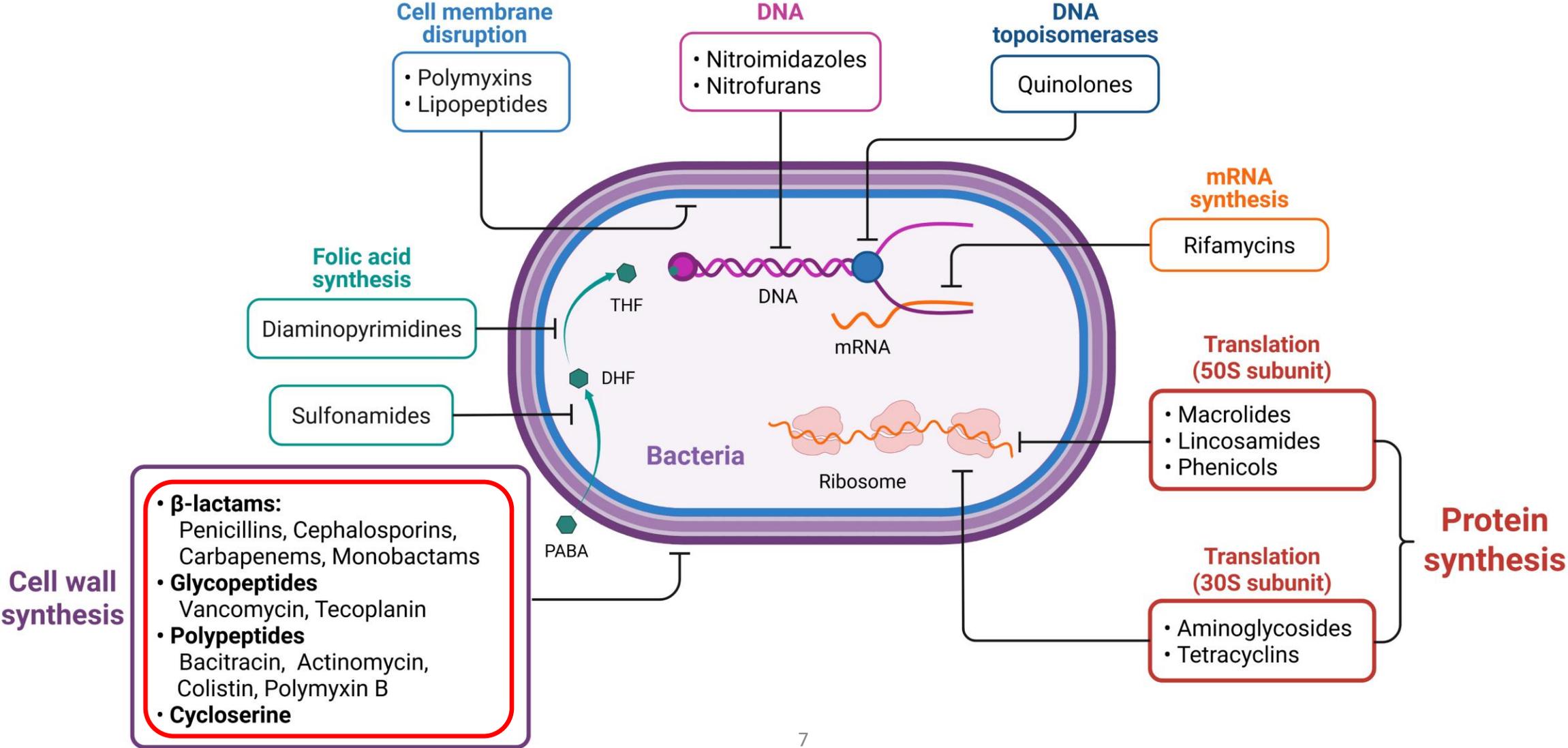
Antimicrobial Therapy Targets

1. **Inhibition of cell wall synthesis:** penicillins, cephalosporins, bacitracin, vancomycin

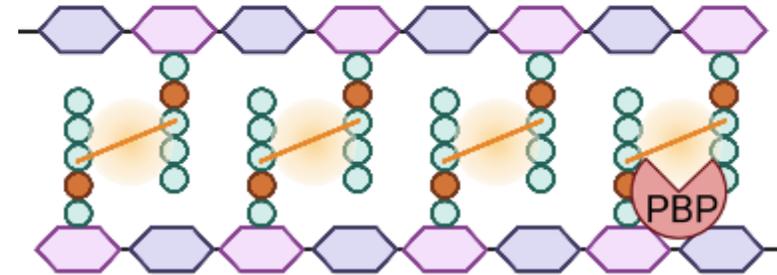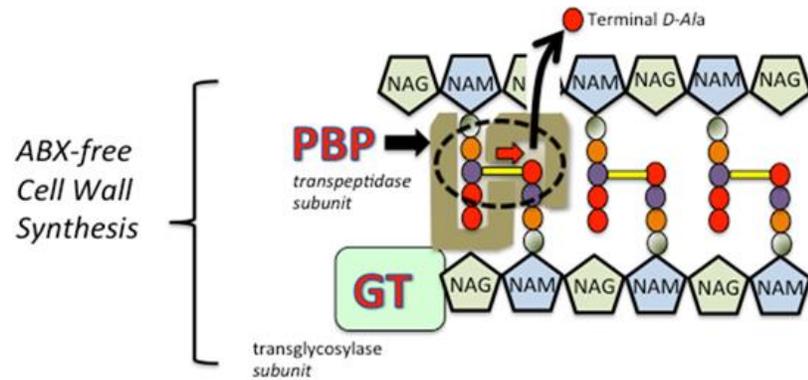2. **Inhibition of protein synthesis:** chloramphenicol, erythromycin, tetracyclines, streptomycin

DNA

mRNA

Protein

Transcription

Translation

Replication

Enzyme

5. **Inhibition of essential metabolite synthesis:** sulfanilamide, trimethoprim

4. **Injury to plasma membrane:** polymyxin B

3. **Inhibition of nucleic acid replication and transcription:** quinolones, rifampin
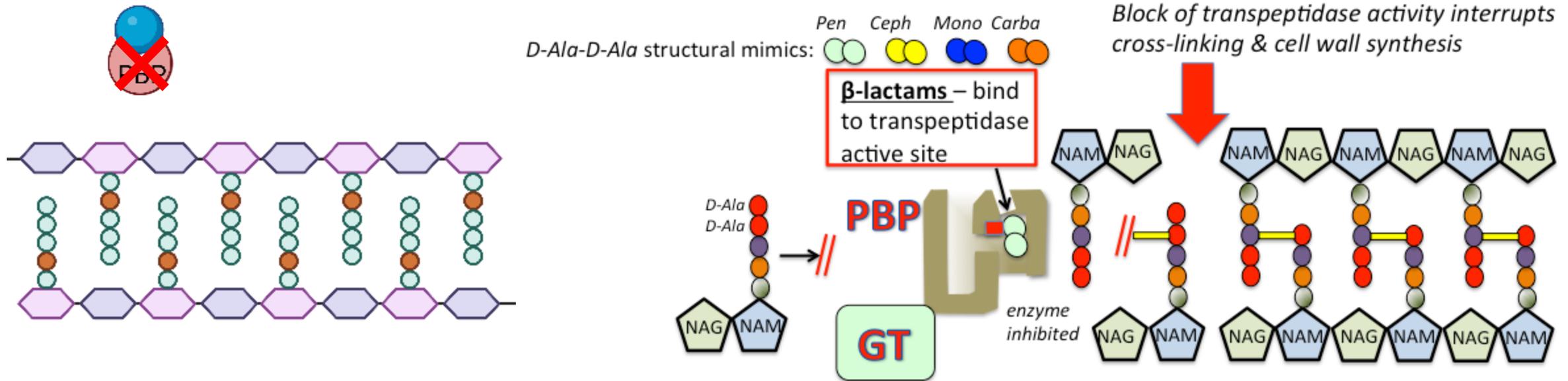
Antimicrobial Therapy Targets

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.
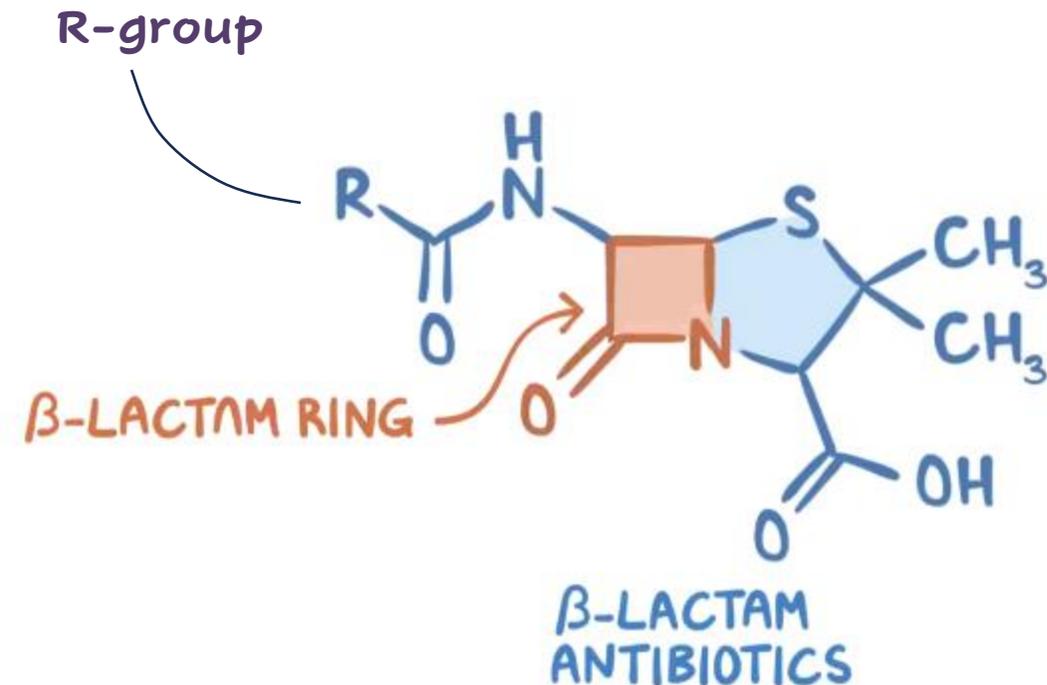
Beta-lactamase destroys beta-lactam ring ☹

- 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

R-group

β-LACTAM RING
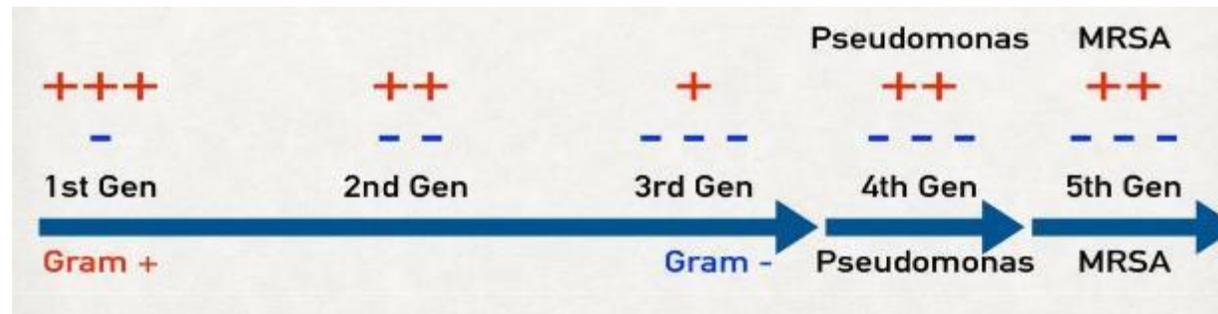
β-LACTAM ANTIBIOTICS
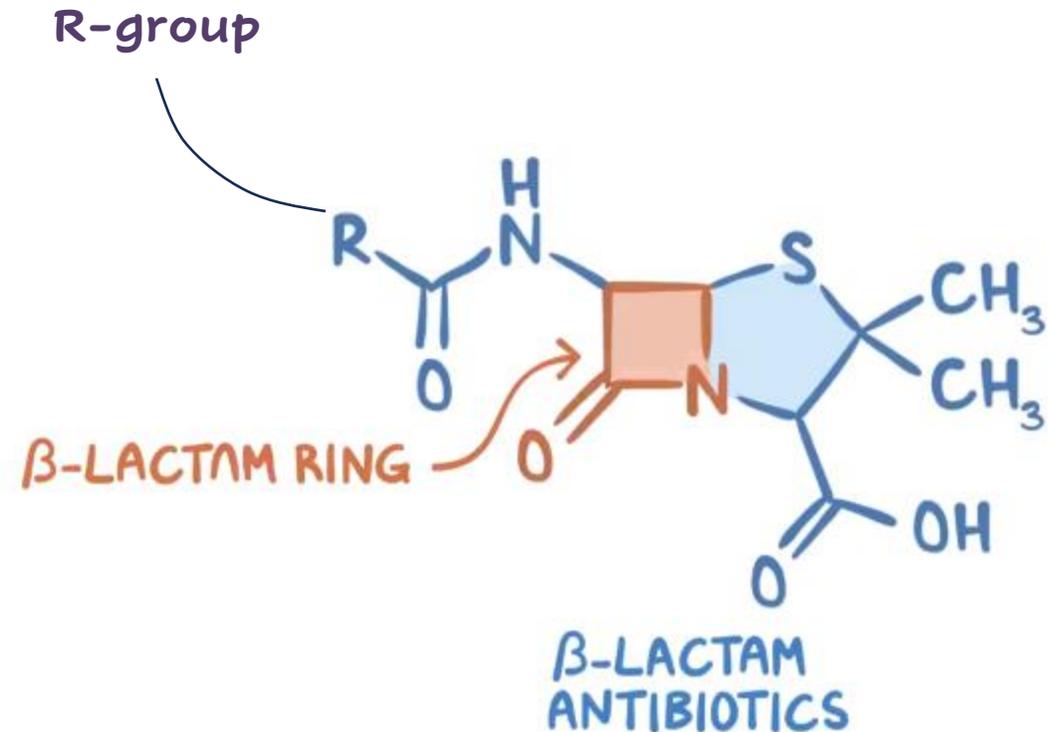
Beta-lactamase inhibitors

- Examples:

  - Clavulanic Acid (i.e. amoxicillin-clavulanate)

  - Sulbactam (i.e. ampicillin-sulbactam)

  - Tazobactam (i.e. piperacillin-tazobactam)

- Bind to beta lactamase and disable them

  - Which allow beta lactams to work

R-group

β-LACTAM RING

β-LACTAM ANTIBIOTICS

Cephalosporins Gram coverage

Gram coverage

			Pseudomonas	MRSA
+++	++	+	++	++
−	− −	− − −	− − −	− − −
1st Gen	2nd Gen	3rd Gen	4th Gen	5th Gen
Gram +		Gram −	Pseudomonas	MRSA

Inhibition of cell wall synthesis

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

① Vancomycin targets bacteria cell wall and binds to two D-alanin residues on the end of the peptide chains

② Vancomycin prevents cross-linking enzyme from binding to residues

③ Vancomycin prevents cell wall strands from cross-linking, leading to cell wall rupture

Bacterial cell wall components

Bacterial cell wall components

Cross-linking enzyme

Vancomycin

| N-acetylglucosamine (NAG) subunit | N-acetylmuramic acid (NAM) subunit | Pentaglycine chain | Alanine (L or D) | D-glutamate | L-lysine |

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

Inhibition of cell wall synthesis

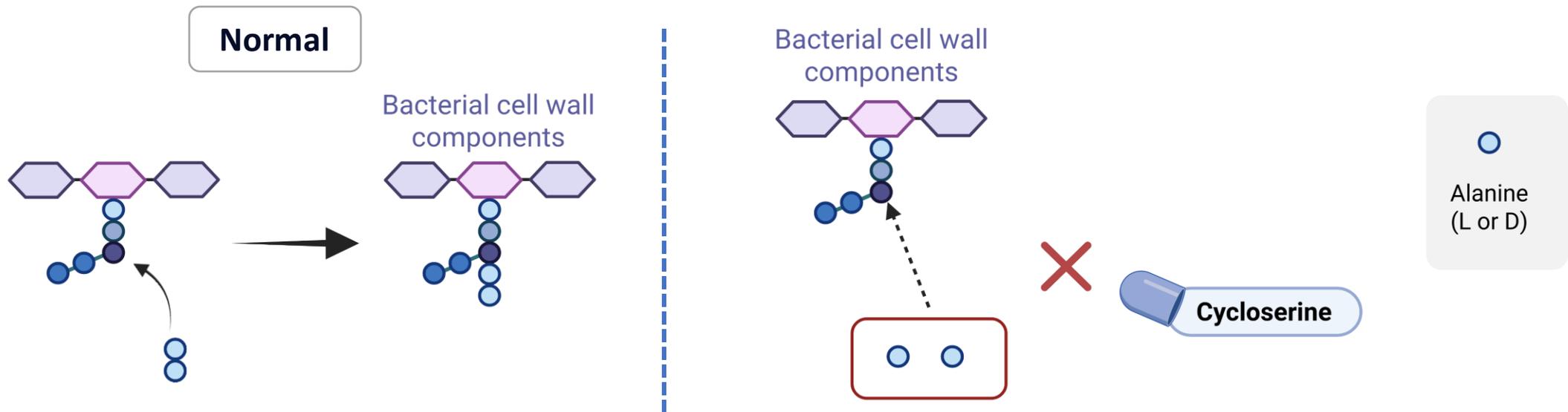**4. In the presence of the drug (Bacitracin):**
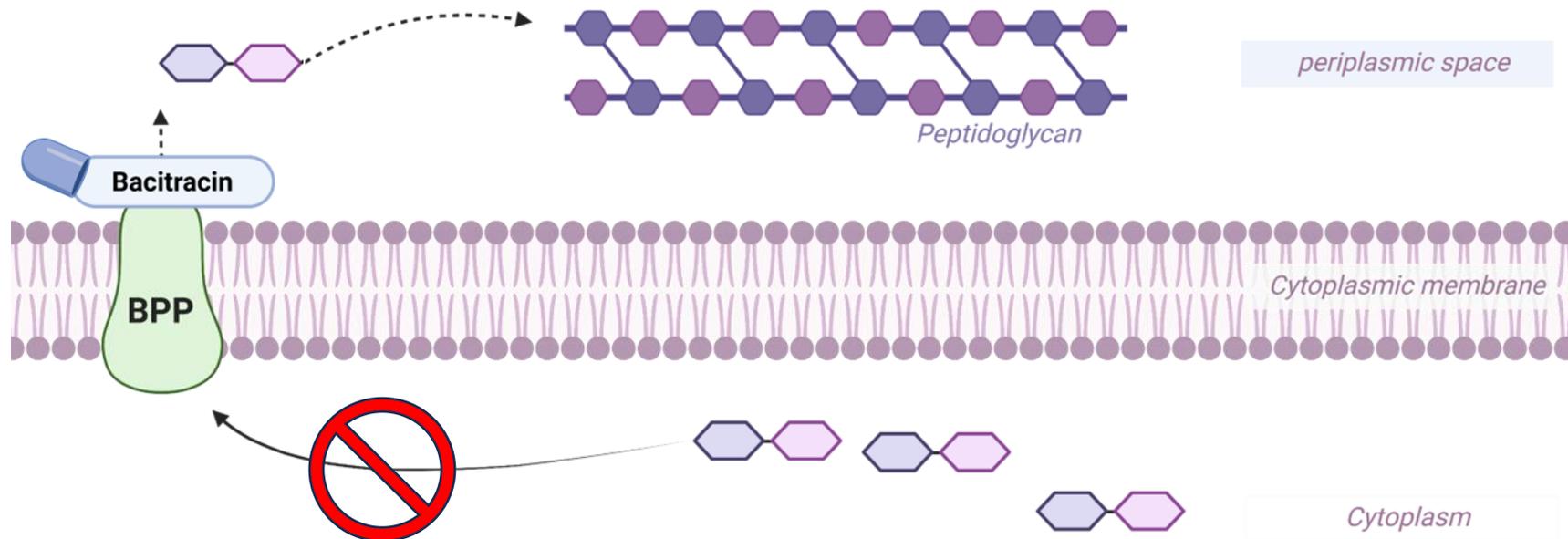
By binding to Bactoprenol phosphate (BPP), Bacitracin prevents the dephosphorylation of this lipid carrier, effectively halting the transport of peptidoglycan precursors (NAG & NAM)

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-alanine ligase, cycloserine blocks the formation of the D-Ala-D-Ala link in peptidoglycan precursors, stopping cell wall synthesis.
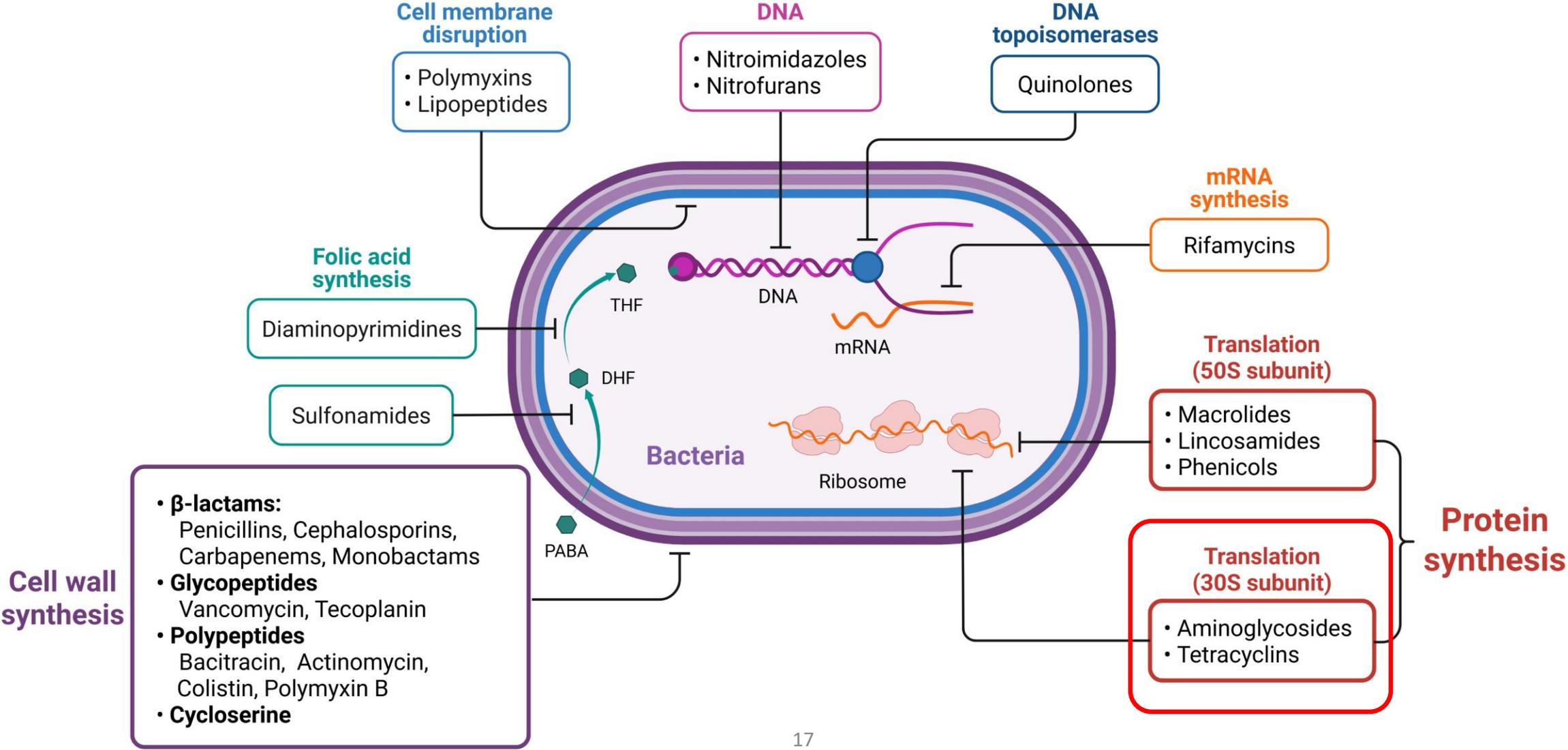
Inhibition of cell wall synthesis

So, no cell wall synthesis → what next?

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

Antimicrobial Therapy Targets

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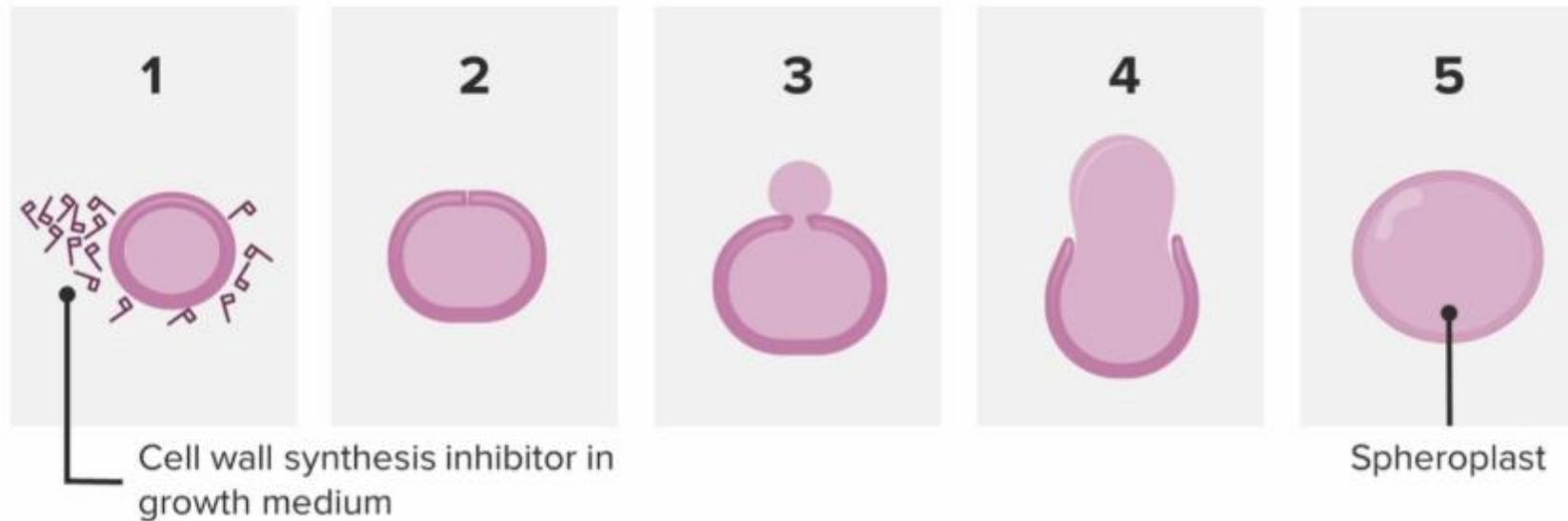
Inhibition of protein synthesis (30S)

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

amino acids

growing polypeptide chain

50S large subunit

tRNA

mRNA

3′

5′

30S small subunit

19
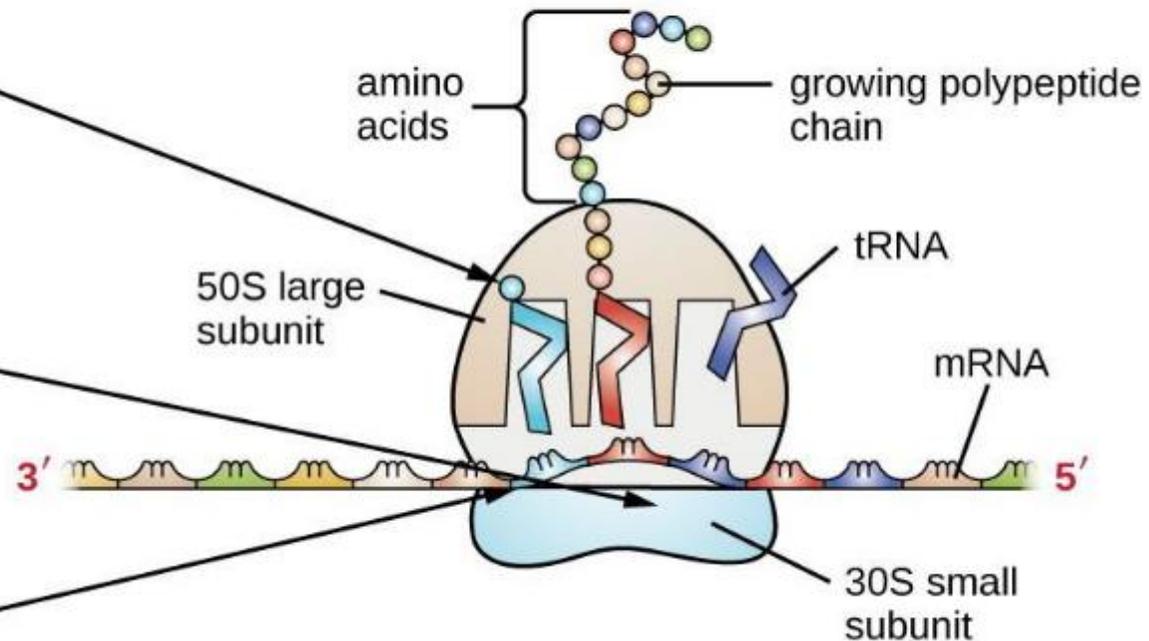
Inhibition of protein synthesis (30S) Aminoglycosides

- 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
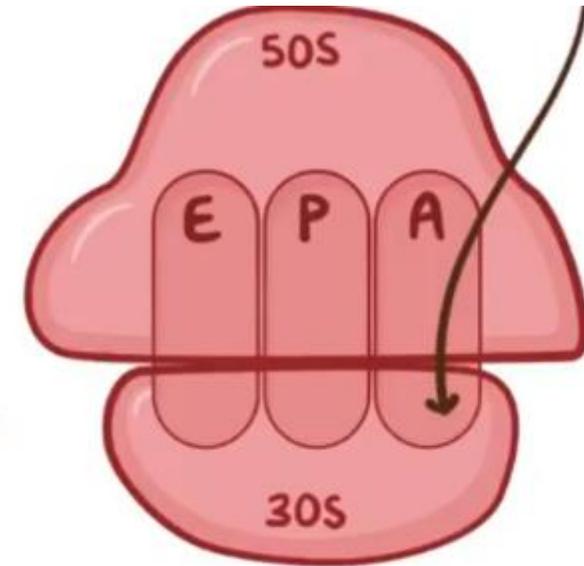- Can work as bacteriostatic or bactericidal (depending on the dose)

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
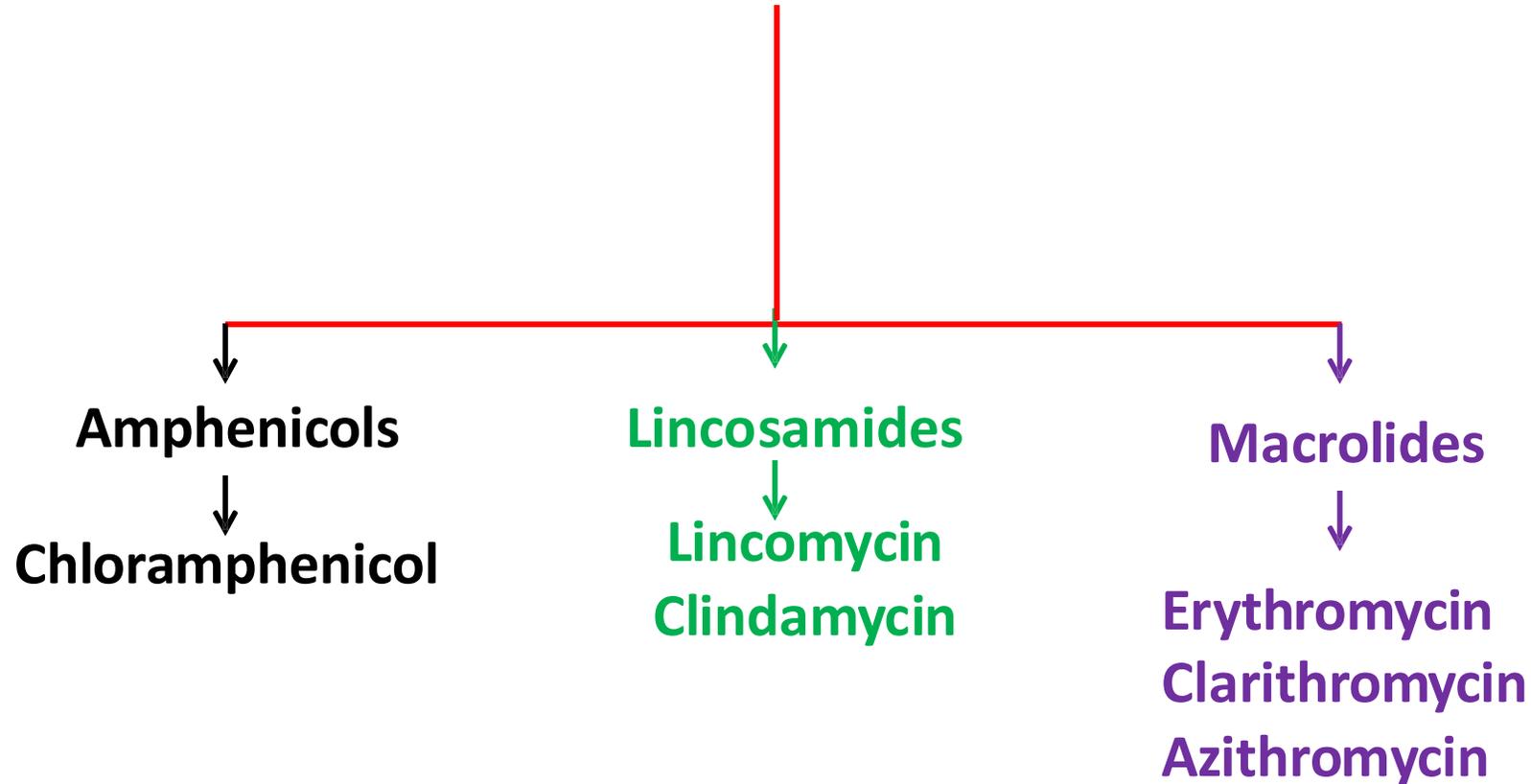
Spectrum of activity - Broad spectrum; Useful against intracellular bacteria
Resistance – Common
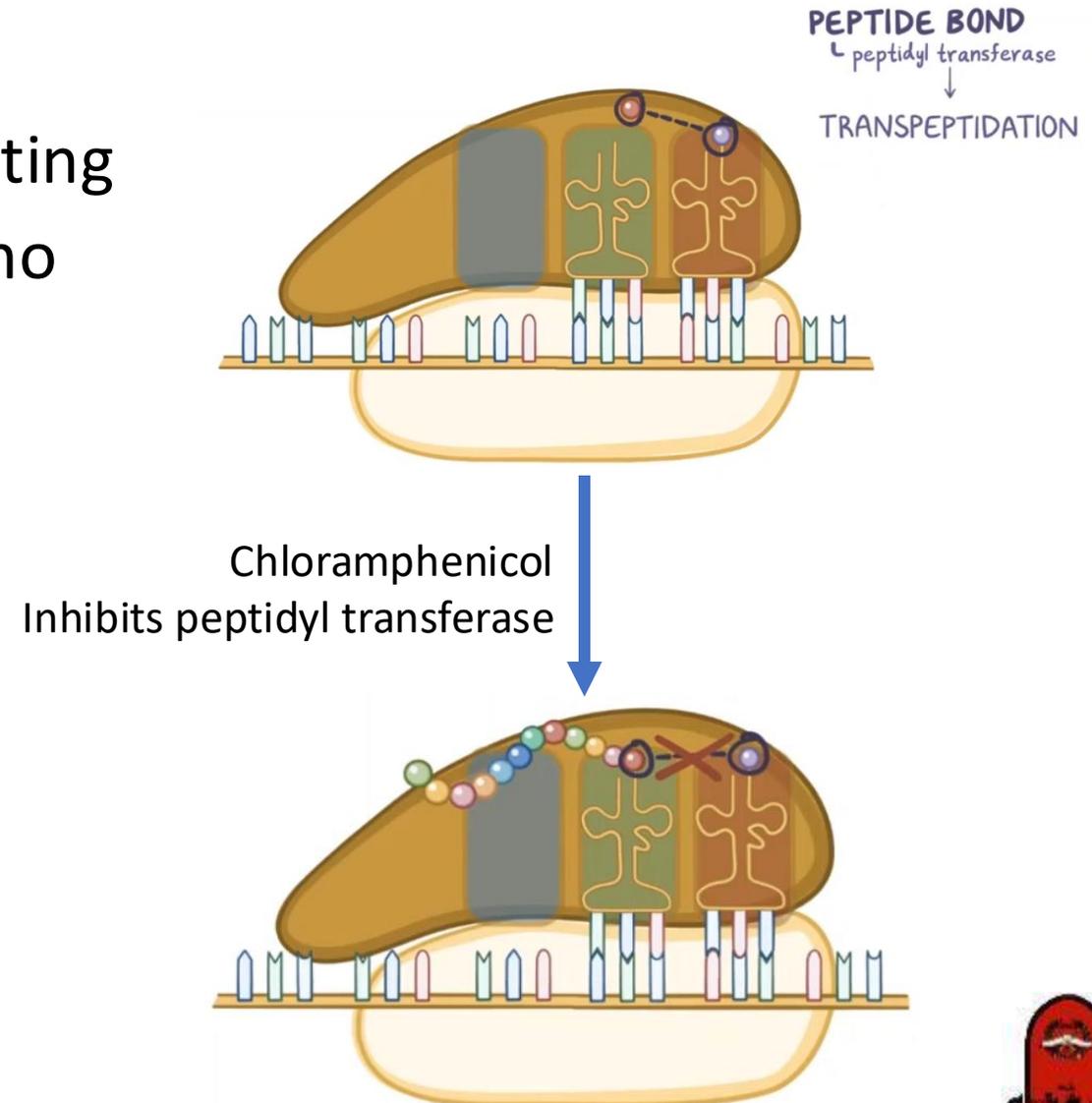
Inhibition of protein synthesis (50S)

Antimicrobials that Bind to the 50S Ribosomal Subunit

Amphenicols	Lincosamides	Macrolides
Chloramphenicol	Lincomycin Clindamycin	Erythromycin Clarithromycin Azithromycin

# Inhibition of protein synthesis (50S)
Chloramphenicol

- Inhibits peptidyl transferase → Preventing peptide bond formation between amino acid and polypeptide chain

- Broad spectrum bacteriostatic drug

PEPTIDE BOND
└ peptidyl transferase
↓
TRANSPEPTIDATION

Chloramphenicol
Inhibits peptidyl transferase

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Inhibition of protein synthesis (50S)
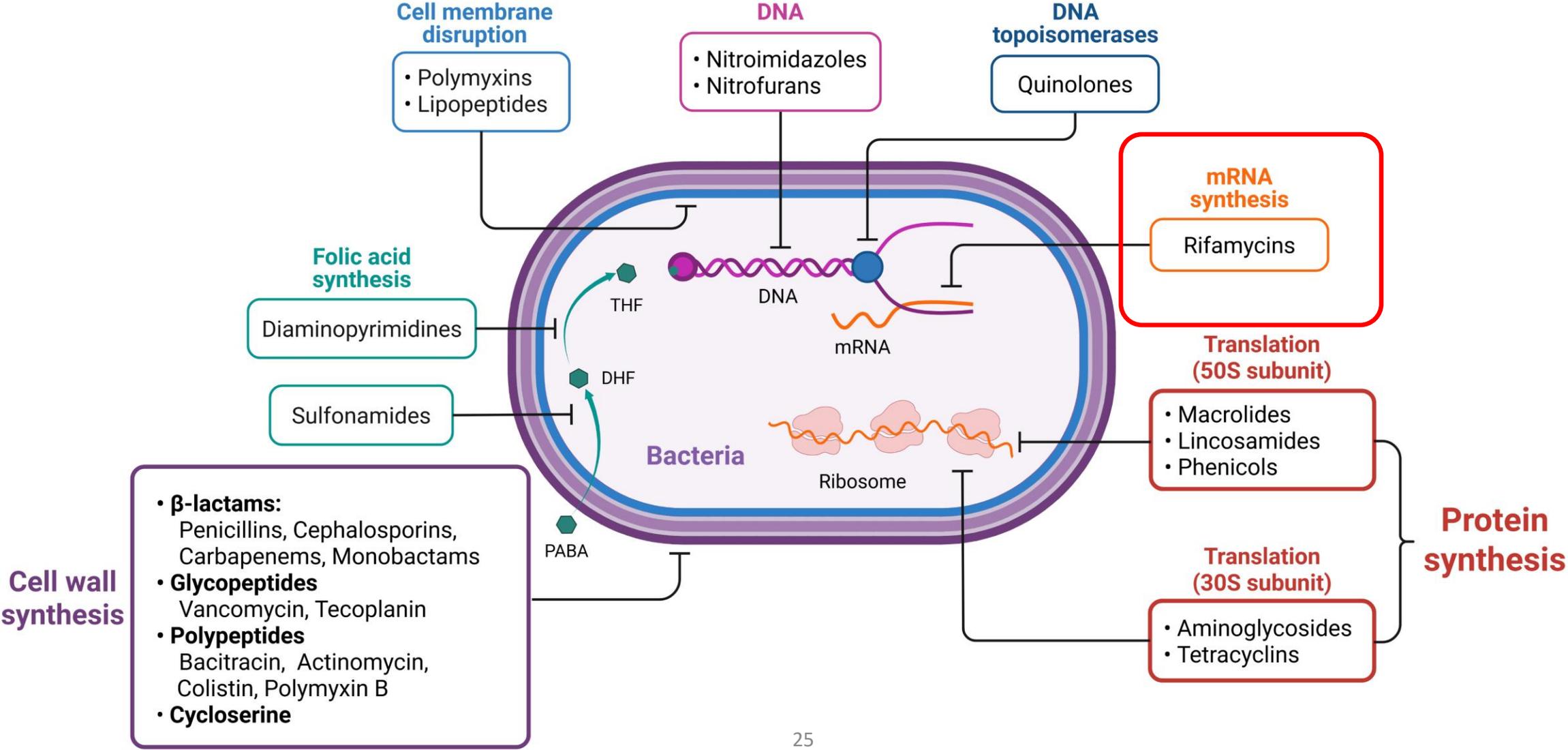## Macrolides & Clindamycin

• Binds to 50s and Block translocation

• Broad spectrum bacteriostatic drug

Drug
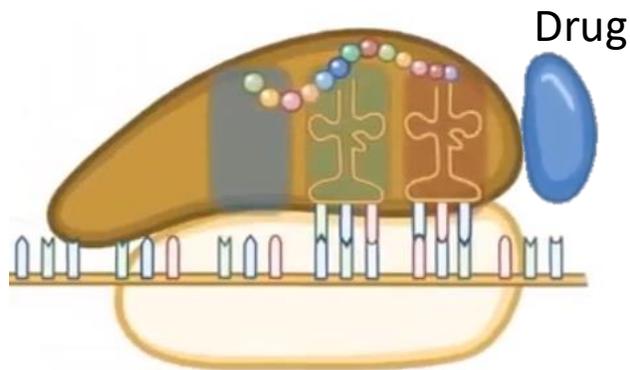
Binds to 50s and Block translocation
So Ribosome can not slide to the next codon on the mRNA
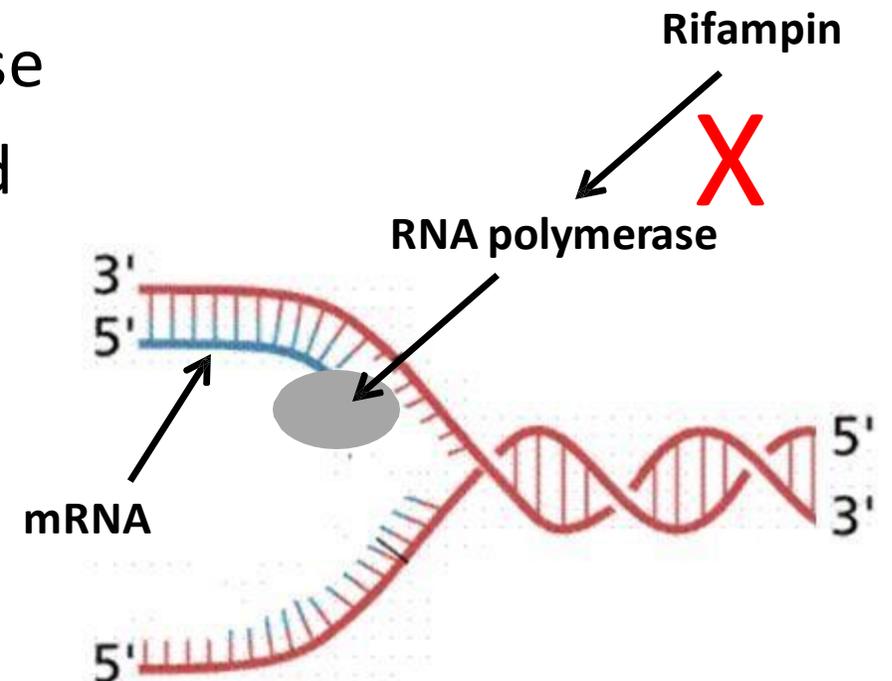
Antimicrobial Therapy Targets
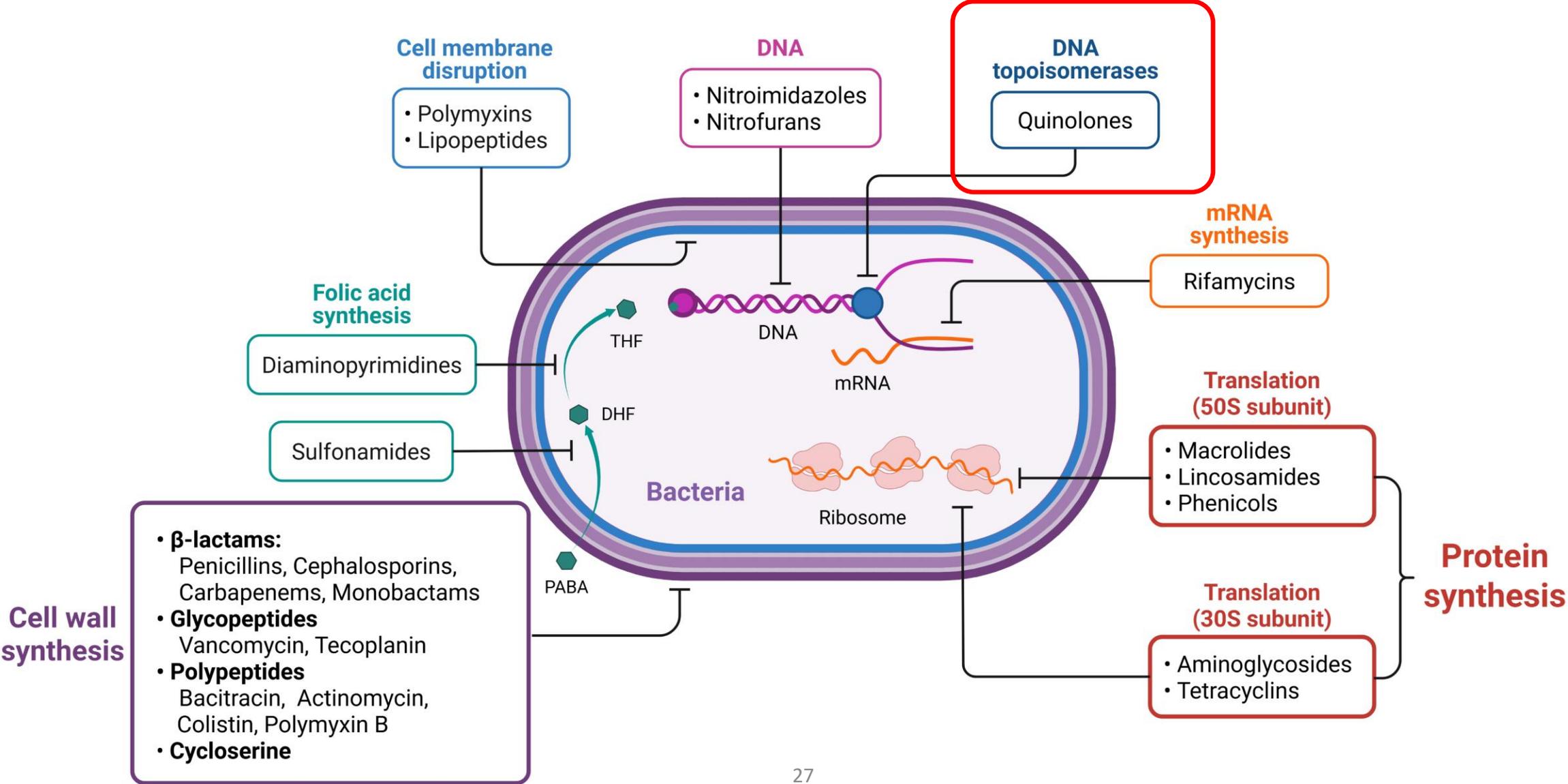
Inhibitors of mRNA Synthesis

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

Rifampin

X

RNA polymerase

mRNA

3'
5'

5'
3'

5'

Antimicrobial Therapy Targets

Cell membrane disruption
- Polymyxins
- Lipopeptides

DNA
- Nitroimidazoles
- Nitrofurans

DNA topoisomerases
- Quinolones

mRNA synthesis
- Rifamycins

Folic acid synthesis
- Diaminopyrimidines
- Sulfonamides

THF

DHF

PABA

DNA

mRNA

Bacteria

Ribosome

Translation (50S subunit)
- Macrolides
- Lincosamides
- Phenicols

Translation (30S subunit)
- Aminoglycosides
- Tetracyclins

Protein synthesis

Cell wall synthesis
- **β-lactams:**
  Penicillins, Cephalosporins, Carbapenems, Monobactams
- **Glycopeptides**
  Vancomycin, Tecoplanin
- **Polypeptides**
  Bacitracin, Actinomycin, Colistin, Polymyxin B
- **Cycloserine**

Inhibitors of DNA Synthesis

Fluoroquinolones:

nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin.

Mode of action - These antimicrobials inhibit DNA gyrase and topoisomerase IV, preventing removal of positive supercoils that accumulate during replication and stabilizing DNA-enzyme complexes with breaks, thereby blocking DNA synthesis and causing bacterial cell death.

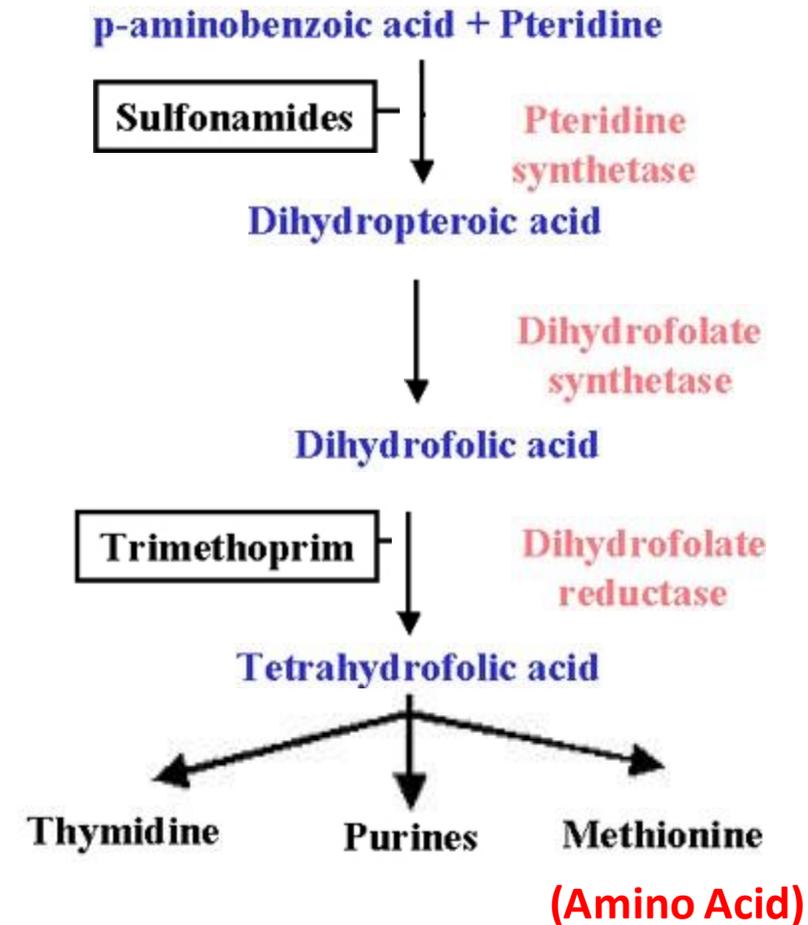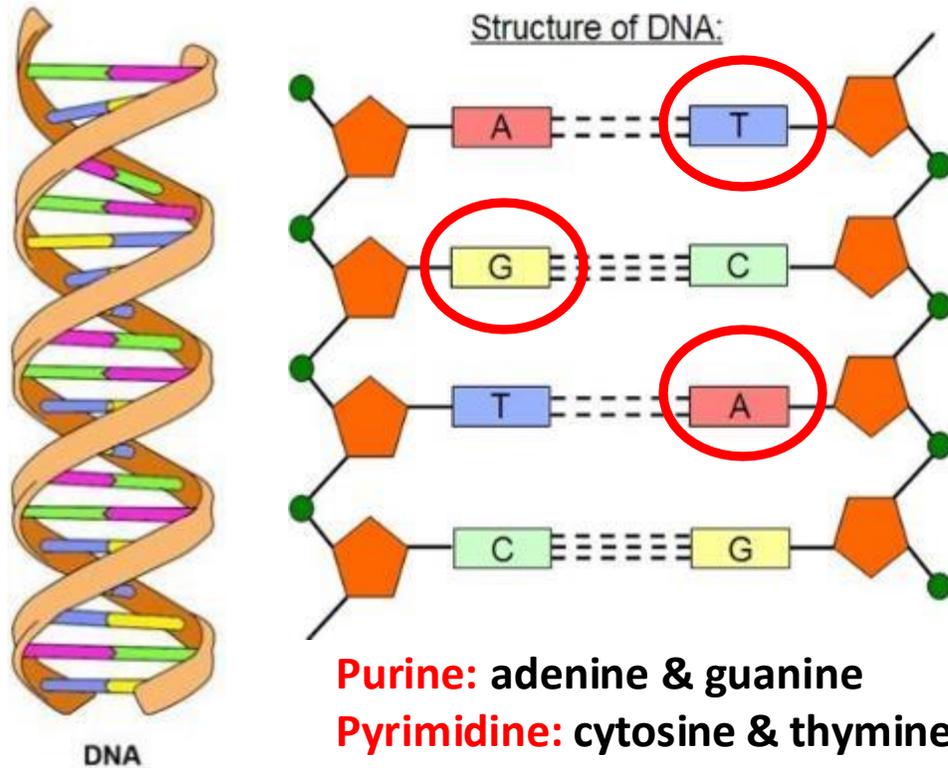**Resistance** - Common for nalidixic acid

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.
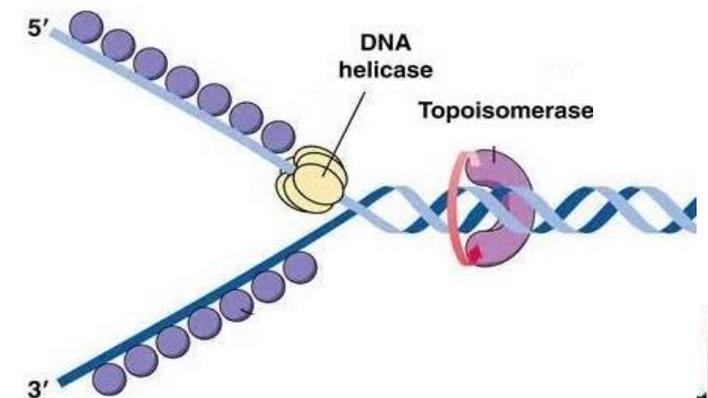
Nucleic acid synthesis is inhibited by:
1. **Trimethoprim**
2. **Sulfonamide group:** Sulfamethoxazole, Sulfadiazine Sulfathiazole, Sulfamerazine

Structure of DNA:

Purine: adenine & guanine
Pyrimidine: cytosine & thymine

DNA

p-aminobenzoic acid + Pteridine

Sulfonamides — Pteridine synthetase

Dihydropteroic acid

Dihydrofolate synthetase

Dihydrofolic acid

Trimethoprim — Dihydrofolate reductase

Tetrahydrofolic acid

Thymidine   Purines   Methionine

(Amino Acid)

- **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)
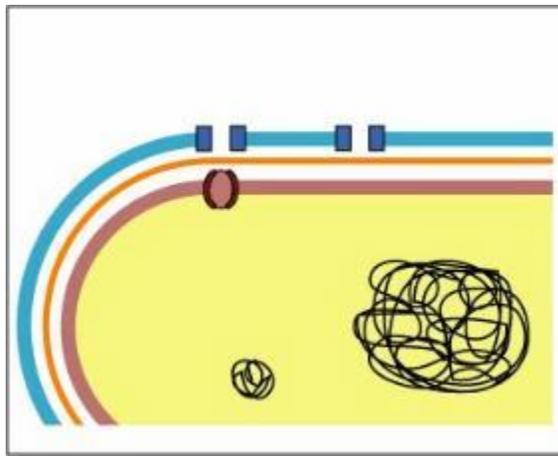
Principles of Antimicrobial Drug Resistance

- **Altered permeability**
  - Altered influx
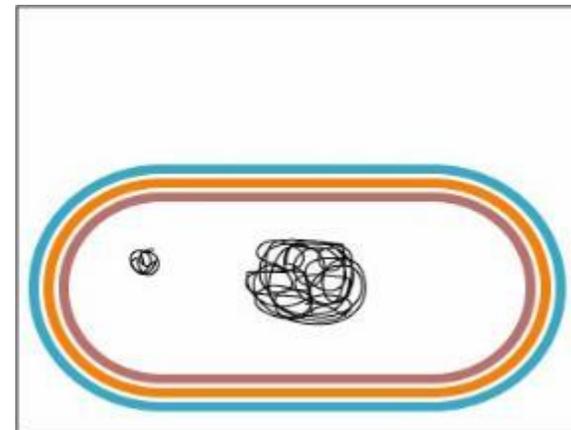   - Gram negative bacteria
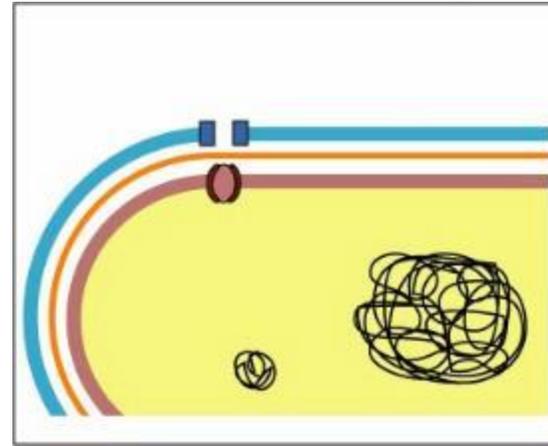
Altered permeability

- Altered efflux
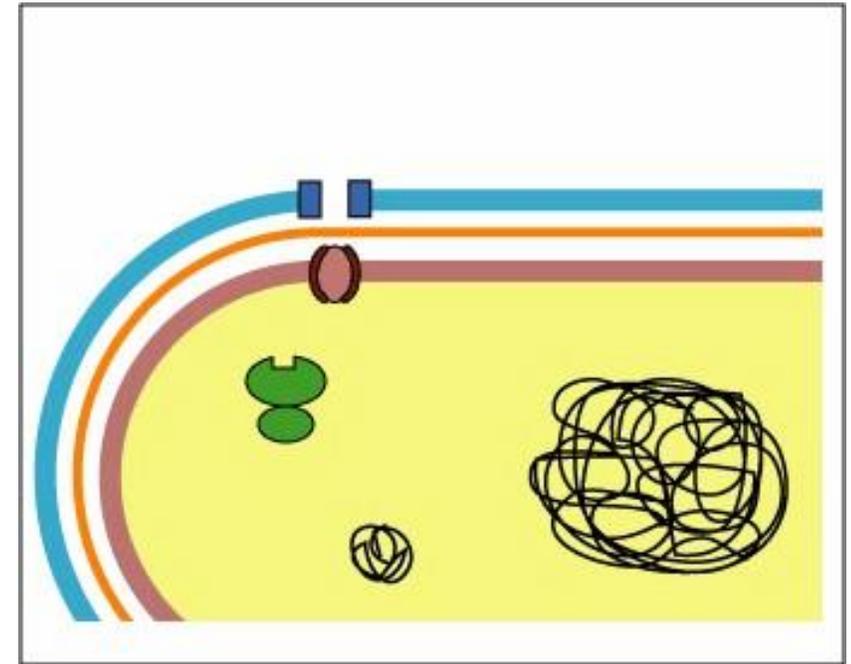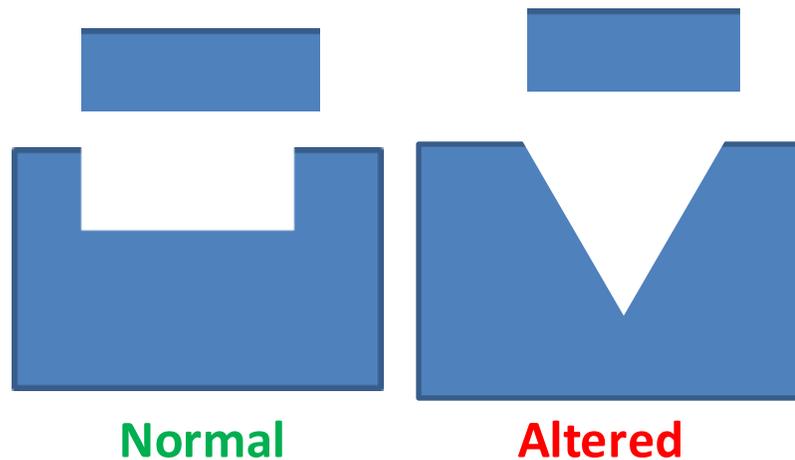 - tetracycline

- Inactivation
  - ➢ Beta-lactamase

Antimicrobial Drug Resistance Principles and Definitions

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

Normal   **Altered**

Question

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

يا بني لقد تعبت... أَتَحمِلُني؟

مثلما كنتَ تحملني يا أَبي،

وسأحمل هذا الحنين

إلى

أَوَّلي وإلى أَوَّلِهْ

وسأقطع هذا الطريق إلى

آخري ... وإلى آخِرِهْ!

حفظكم الله وحفظ لكم أهاليكم