# PROTEIN SYNTHESIS INHIBITORS

**mRNA + ribosome (30S and 50S) ⟶ protein**

**Protein synthesis can be inhibited at several steps.**

**1. 30S subunit:**
**AMINOGLYCOSIDES**
**TETRACYCLINES**

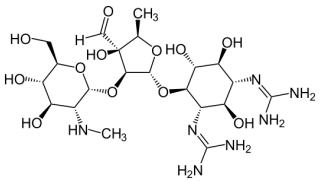
**2. 50S subunit:**
**CHLORAMPHENICOL,**
**ERYTHOMYCIN (MACROLIDE ANTIBIOTICS)**
**(AZITHROMYCIN)**

## AMINOGLYCOSIDES(氨基甙类抗生素)

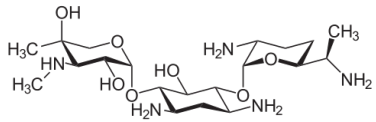
**e. g Streptomycin, Neomycin, Kanamycin, Gentamycin, Tobramycin, Amikacin**

An **aminoglycoside** is a molecule composed of amino-modified sugars.

Usually produced from *Streptomyces* genus (suffix *–mycin)*, or from *Micromonospora* (suffix *–micin)*



**Streptomycin**



**Gentamicin**

## AMINOGLYCOSIDES

### Mechanism of action

- **Bind irreversibly to 30S subunit of bacterial ribosome. Inhibit ribosomal translocation where the peptidyl-tRNA moves from the A-site to the P-site.**
- **Interfere with the proofreading process, causing increased rate of error in protein synthesis with premature termination, producing toxic proteins due to mistranslation.**
- **Mistranslated toxic proteins perturb cell membrane and respiratory chain, which produces reactive oxygen species that kill cells.**
- **Disrupt the integrity of bacterial cell membrane.**
- **Bactericidal possibly because production of ROS or disruption of cell membrane (not due simply to inhibition of translation since other translational inhibitors are only bacteriostatic)**

## AMINOGLYCOSIDES

### Mechanism of resistance

- **Reduced uptake or decreased cell permeability (usually chromosomally mediated, resulting in cross-reactivity to all aminoglycosides, usually confers moderate levels of resistance)**
- **Alterations at the ribosomal binding sites (rRNA mutations, important for agents that binds a single site of the ribosome (e.g. streptomycin), less significant for agents that bind multiple sites because a single-step mutation won't be enough to confer resistance).**
- **Production of aminoglycoside modifying enzymes (the most common type of aminoglycoside resistance. Over 50 different enzymes have been identified, which are usually carried on plasmids and transposons.**

**Three major classes of enzymes are involved:**

**1. N-Acetyltransferases (AAC) – catalyzes acetyl CoA-dependent acetylation of an amino group**

**2. O-Adenyltransferases (ANT) – catalyzes ATP-dependent adenylation of hydroxyl group**

**3. O-Phosphotransferases (APH) – catalyzes ATP-dependent phosphorylation of a hydroxyl group**

## AMINOGLYCOSIDES

### Mechanism of resistance

**Examples of modifying enzyme-based aminoglycoside resistance phenotypes of *Enterobacteriaceae spp.*, including *E. coli* (excluding *Serratia spp.* and *Klebsiella spp.*)**

classical= historic phenotype of the species, without acquired resistance

| Phenotype | classical | AAC(3)I | AAC(3)II | AAC(3)IV | AAC(6') | ANT(2') | APH(3') |
|---|---|---|---|---|---|---|---|
| Gentamicin | S | R | R | R | S/r | R | S |
| Netilmicin | S | S | R | R | R | S | S |
| Tobramycin | S | S | R | R | R | R | S |
| Amikacin | S | S | S | S | R | S | S |
| Kanamycin | S | S | R | r | R | R | R |
| Neomycn | S | S | S | R | R | S | R |

## AMINOGLYCOSIDES

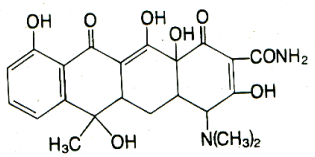
### Clinical indications

- **Severe, hospital-acquired infections with multidrug resistant Gram negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter*, *Enterobacter,* Serratia marcescens(粘质沙雷氏菌), and Providencia stuartii(产碱普罗威登斯菌).**
- **Non-tubercular mycobacterial infections and tuberculosis (if caused by sensitive strains) when first line drugs fail to control the infection.**
- **May be combined with a beta-lactam( $\beta$ -内酰胺 ) antibiotic for empiric therapy(经验疗法) for people with neutropenia(嗜中性白血球减少症) and fever.**
- **High doses have adverse effects (hearing loss and kidney damage, low therapeutic index).**
- **Poor oral absorption and thus mostly given in iv or im.**

## TETRACYCLINEs

- A broad-spectrum polyketide antibiotic produced by the *Streptomyces* genus of actinobacteria
- A protein synthesis inhibitor indicated for use against many bacterial infections.
- Effective only against rapidly dividing cells,
- Bacteriostatic

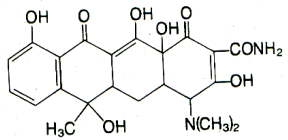
TETRACYCLINE



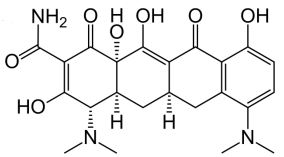
## Major derivatives of TETRACYCLINEs

**Tetracycline, minocycline, doxycycline, tigecycline**

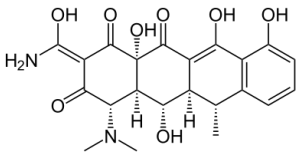
TETRACYCLINE



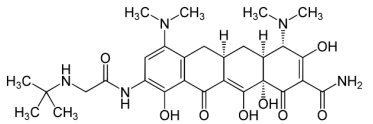
Minocycline



Doxycycline

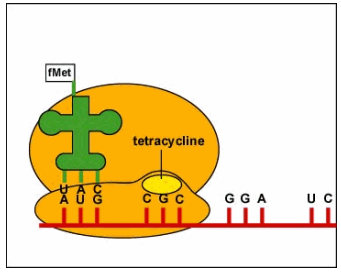


Tigecycline



## Tetracycline Mechanism of Action

- Broad spectrum antibiotics
- Binds reversibly to 30S ribosomal subunits, blocking the binding of aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex, preventing introduction of new amino acids to the nascent peptide chain, reversible upon drug removal.




## TETRACYCLINEs

### Mechanisms of Resistance

Resistance is usually acquired through horizontal transfer of a gene that either encodes an **efflux pump** or a **ribosomal protection protein**.

Efflux pumps actively eject tetracycline from the cell, preventing the buildup of an inhibitory concentration of tetracycline in the cytoplasm.

Ribosomal protection proteins interact with the ribosome and dislodge tetracycline from the ribosome, allowing for translation to continue.

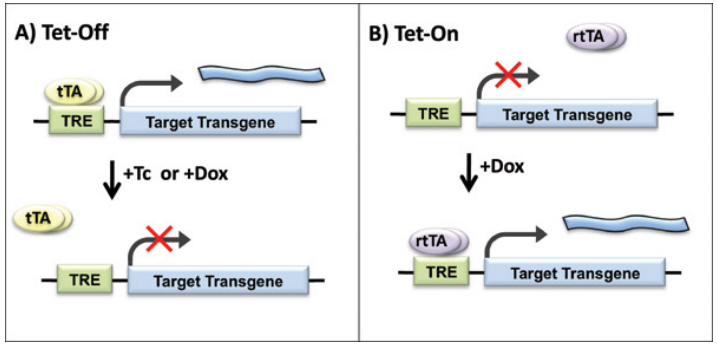
Enzymatic inactivation of tetracycline is the rarest type of resistance, where an acetyl group is added to the molecule, causing inactivation of the drug.

## Tetracyclines

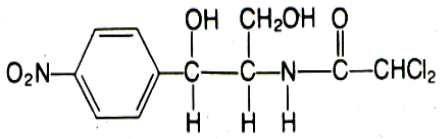
### Clinical indications

- treatment of infections of the urinary tract and the intestine
- treatment of moderately severe acne and rosacea
- treatment of choice for infections caused by chlamydia (trachoma, psittacosis, salpingitis, urethritis, and *L. venereum* infection), Rickettsia (typhus, Rocky Mountain spotted fever), brucellosis, and spirochetal infections (borreliosis, syphilis, and Lyme disease).
- may also be used to treat anthrax, plague, tularemia, and Legionnaires' disease.
- treatment of teeth and gum infections since it tends to concentrate in bones, also used as a bone growth marker.
- Used for transcription activation/inhibition (tet-on/tet off system)

## Use of tetracycline in controled gene expression



## CHLORAMPHENICOL



**Very broad spectrum, active against Gram-positive bacteria (including most strains of MRSA), Gram-negative bacteria, and anaerobes, not active against *Pseudomonas aeruginosa*, Chlamydiae, or *Enterobacter* species, some activity against *Burkholderia pseudomallei***

**Bacteriostatic, not used in combination with bacteriocidal antibiotics**

**Two forms of bone marrow toxicity: bone marrow suppression (usually reversible), and aplastic anemia (rare but generally fatal)**

## CHLORAMPHENICOL

## Mechanism of action

Stops bacterial growth by inhibiting protein synthesis.

Prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome.

It specifically binds to A2451 and A2452 residues in the 23S rRNA of the 50S ribosomal subunit, preventing peptide bond formation.

While chloramphenicol and the macrolide both interact with ribosomes, chloramphenicol is not a macrolide. It directly interferes with substrate binding, whereas macrolides sterically block the progression of the growing peptide.

## CHLORAMPHENICOL

## Mechanism of resistance

Three mechanisms of resistance to chloramphenicol:

reduced membrane permeability (very easy to select, most common mechanism of low-level chloramphenicol resistance)

mutation of the 50S ribosomal subunit (rare)

elaboration of chloramphenicol acetyltransferase (CAT usually mediates high-level resistnce by inactivating chloramphenicol via covalently linking one or two acetyl groups, derived from acetyl-S-coenzyme A, to the hydroxyl groups on the chloramphenicol molecule. The acetylation prevents chloramphenicol from binding to the ribosome.

resistance may be carried on a plasmid that also codes for resistance to other drugs

## CHLORAMPHENICOL

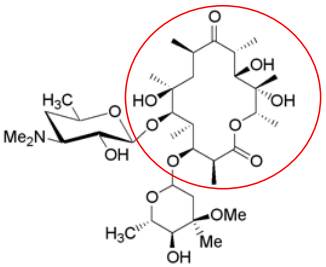
## Clinical indications

Excellent BBB penetration makes chloramphenical the first choice treatment for staphylococcal brain abscesses.

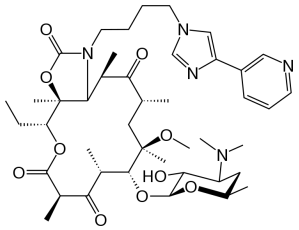
treatment of brain abscesses due to mixed organisms or when the causative organism is not known.

Chloramphenicol is active against the three main bacterial causes of meningitis: *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*.

# Macrolide antibiotics



Erythromycin



Telithromycin

A group of drugs whose activity stems from the presence of a *macrolide ring*, a large macrocyclic lactone ring to which one or more deoxy sugars may be attached.

The lactone rings are usually 14-, 15-, or 16-membered. Macrolides belong to the polyketide class of natural products.

# Macrolide Mechanism of Action

binding to the 50s subunit of the bacterial 70s rRNA complex

interfering with aminoacyl translocation, preventing the transfer of the tRNA bound at the A site of the rRNA complex to the P site of the rRNA complex.

the A site remains occupied and, thus, the addition of an incoming tRNA and its attached amino acid to the nascent polypeptide chain is inhibited.

This interferes with the production of functionally useful proteins, which is the basis of this antimicrobial action.

# Macrolides

## Mechanisms of Resistance

The primary resistance to macrolides occurs by post-transcriptional methylation of the 23S bacterial ribosomal RNA. This acquired resistance can be either plasmid-mediated or chromosomal, i.e., through mutation, and results in cross-resistance to macrolides, lincosamides, and streptogramins (an MLS-resistant phenotype).

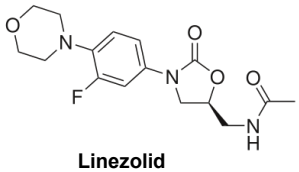
Two other types of acquired resistance rarely seen include the production of drug-inactivating enzymes (esterases or kinases), as well as the production of active ATP-dependent efflux proteins that transport the drug outside of the cell.

# Macrolides

## Clinical indications

Macrolides are active against:
Aerobic and anaerobic Gram-positive cocci, except for most enterococci, many *Staphylococcus aureus* strains (especially methicillin-resistant strains), and some *Streptococcus pneumoniae* and *S. pyogenes* strains
Mycoplasma pneumoniae
Chlamydia trachomatis
Chlamydophila pneumoniae
Legionella sp
Corynebacterium diphtheriae
Campylobacter sp
Treponema pallidum
Propionibacterium acnes
Borrelia burgdorferi

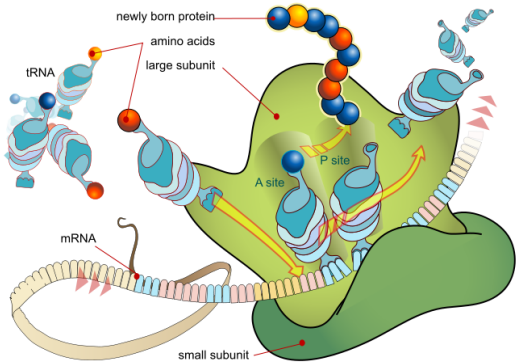
## Oxazolidinones (恶唑烷酮)



**Linezolid**

**Linezolid (利奈唑胺)** is a newly approved synthetic antibiotic used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several other antibiotics.

## Oxazolidinones: Mechanism of action




Linezolid inhibits the first step of protein synthesis, *initiation*, by preventing the formation of the *initiation complex*, composed of the 30S and 50S subunits of the ribosome, tRNA, and mRNA.

## Oxazolidinones: Mechanism of Resistance

No cross-resistance with other protein synthesis inhibitors due to its unique mode of action

The *intrinsic* resistance of most Gram-negative bacteria to linezolid is due to the activity of efflux pumps, which actively "pump" linezolid out of the cell faster than it can accumulate.

Gram-positive bacteria usually develop resistance to linezolid as the result of a point mutation known as *G2576T*, in which a guanine base is replaced with thymine in base pair 2576 of the genes coding for 23S ribosomal RNA. This is the most common mechanism of resistance in staphylococci, and the only one known to date in isolates of *E. faecium* (屎肠球菌).

Other mechanisms have been identified in *Streptococcus pneumoniae* (including mutations in an RNA methyltransferase that methylates G2445 of the 23S rRNA and mutations causing increased expression of ABC transporter genes and in *Staphylococcus epidermidis*.

## Oxazolidinones: Clinical indications

Treatment of severe infections caused by Gram-positive bacteria that are resistant to other antibiotics (**Skin and soft tissue infections, Pneumonia)**

**Spectrum:** *Enterococcus faecium, Enterococcus faecalis* (including vancomycin-resistant enterococci), *Staphylococcus aureus* (including methicillin-resistant *Staphylococcus aureus*, MRSA), *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, the *viridans* group streptococci, *Listeria monocytogenes*, and *Corynebacterium* species.

## Folate (叶酸) synthesis inhibitors

Dihydropteroate diphosphate(磷酸二氢喋呤) + PABA(对氨基苯甲酸)

Dihydropteroate Synthetase(二氢喋呤合成酶) ✗ Sulfonamides(磺胺类)

↓

Dihyropteroic acid

↓

Dihydrofolic acid(二氢叶酸)

Dihydrofolate reductase ✗ trimethoprim

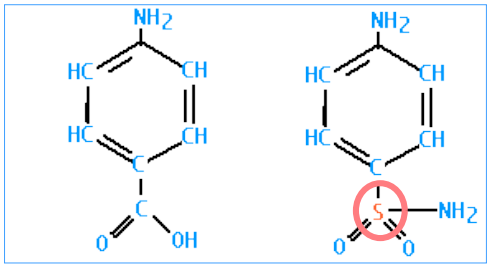
↓

Tetrahydrofolic acid(四氢叶酸)

↓

Nucleotides and Amino Acids

## Sulfanilamide is a substrate analog of PABA



PABA Sulfanilamide

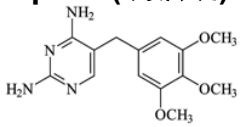
para-aminobenzoic acid(对氨基苯（甲）酸)

Bacteria-specific **PABA → Folate**

**Reduction in purines, pyrimidines, and amino acids**

## DHFR(二氢叶酸还原酶) Inhibitor:

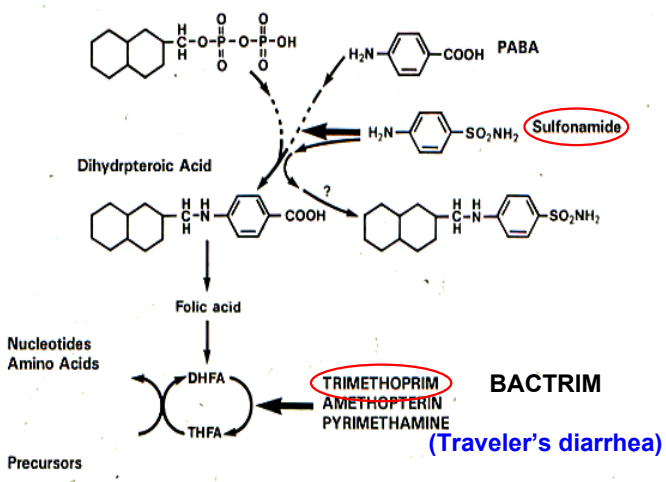
**Trimethoprim (甲氨苄啶)**



**DHFR inhibition: tetrahydrofolate ↓**

**Blocks synthesis of purines, pyrimidines and amino acids.**

## Synergism of sulfanilamide and trimethoprim

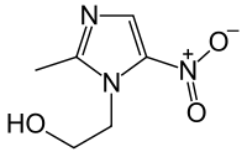



**BACTRIM**

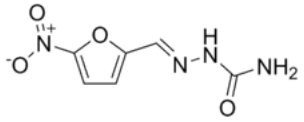
**(Traveler's diarrhea)**

## Others: metronidazole(灭滴灵), nitrofuran(硝基呋喃)

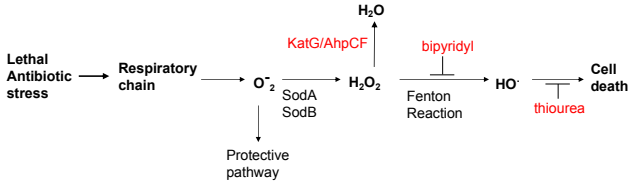
**Metronidazole: useful for** anaerobic bacteria and protozoa. It is the drug of choice for first episodes of mild-to-moderate *Clostridium difficile* infection. Dificid (fidaxomicin) was approved in 2011 for *C. difficile* infection



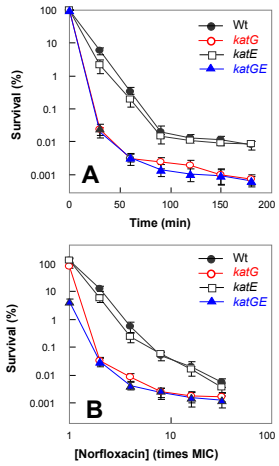
**Nitrofurans:** a class of antimocrobials containing a furan ring with a nitro group, bactericidal by damaging bacterial DNA, protein, and other macromolecules with its highly reactive reduced form.



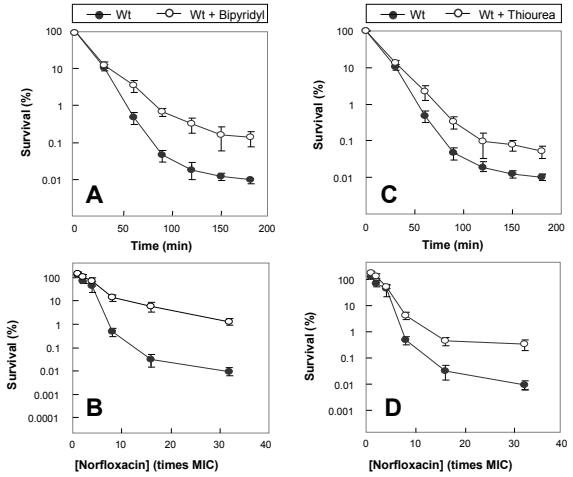
## Oxidative stress: a common pathway for antimicrobial-mediated cell death



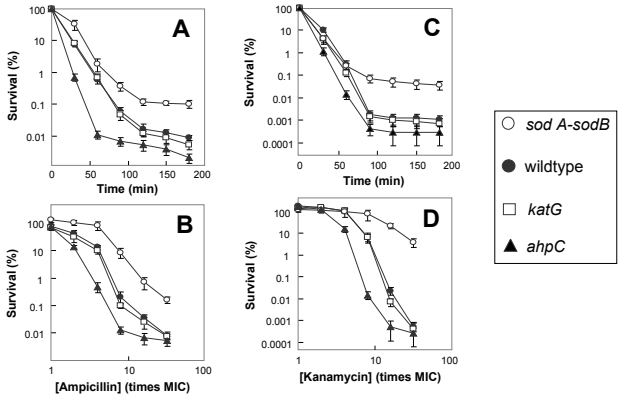

## Catalase/peroxidase deficiency increases norfloxacin-mediated lethality




## Bipyridyl + thiourea (B&T) reduces norfloxacin-mediated lethality



## Effect of ROS scavenging enzyme gene deficiency on antimicrobial lethality




## Home work

1. Name a major resistance mechanism for each protein synthesis inhibitor antimicrobials.
2. Why is tetracycline good for treatment of tooth and gum infections?
3. Why are sulfanilamide and trimethoprim synergistic in treating bacterial infections?