Antibacterial Drugs
# Antibiotics: Commercial Aspects

Table: Top 10 antibacterial companies by global sales of antibiotics in 2004 (Source: Wood Mackenzie\(^{69}\)).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Company</th>
<th>US $ million</th>
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<tr>
<td>1</td>
<td>Pfizer</td>
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<td>2</td>
<td>GlaxoSmithKline</td>
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<td>3</td>
<td>Abbott</td>
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<td>4</td>
<td>Bayer</td>
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<td>5</td>
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<td>Hoffmann-La Roche</td>
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<td>7</td>
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<td>8</td>
<td>Merck &amp; Co.</td>
<td>704</td>
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<td>9</td>
<td>Daiichi</td>
<td>687</td>
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<td>10</td>
<td>Shionogi</td>
<td>678</td>
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<td>Others</td>
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<td>12064</td>
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<tr>
<td>Total</td>
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![Graph showing new antibacterial agents approved in the US (1983-2005)](image)
- Bacteriostatics: Inhibit cell growth and cell division
- Bactericidals: Kill bacteria

Global sales of the major antibacterial classes in 2004 (from Wood Mackenzie[^69]).
Classification of bacteria according to shape

Cocci
- coccus
- diplococci
- diplococci encapsulated (Pneumococcus)
- Staphylococci
- sarcina
- tetrad
- Staphylococci

Bacilli
- coccobacillus
- bacillus
- diplobacilli
- palisades
- Streptobacilli

Others
- Budding and appendaged bacteria
  - hypha
  - stalk
-
- enlarged rod (Fusobacterium)
- Vibrio
- Comma's form (Bdellovibrio)
- Club Rod (corynbacteriaceae)
- Helical form (Helicobacter pylori)
- Corkscrew's form (Borrelia burgdorferi)
- Filamentous
- spirochete

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Infectious Disease</th>
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<tr>
<td>Staphylococcus aureus</td>
<td>skin and wound infection, abscess, bacteremia, nosocomial pneumonia, endocarditis, toxic shock syndrome</td>
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<tr>
<td>Streptococcus pneumoniae</td>
<td>upper respiratory infection, pneumonia, otitis, sinusitis, meningitis</td>
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<tr>
<td>Streptococcus pyogenes</td>
<td>pharyngitis, tonsillitis, skin and soft-tissue infection, scarlet fever</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>bacteremia, endocarditis, urinary-tract infection, peritonitis</td>
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<tr>
<td>Enterococcus faecium</td>
<td>bacteremia, endocarditis, peritonitis</td>
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<tr>
<td>Escherichia coli</td>
<td>bacteremia, urinary-tract and gastrointestinal infection</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>hospital-acquired pneumonia, bacteremia</td>
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<tr>
<td>Proteus spp.</td>
<td>urinary-tract infection</td>
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<tr>
<td>Haemophilus influenzae</td>
<td>respiratory infection, otitis, sinusitis, meningitis</td>
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<tr>
<td>Moraxella catarrhalis</td>
<td>respiratory infection</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td>nosocomial pneumonia, burn infection, bacteremia</td>
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<tr>
<td>Acinetobacter spp.</td>
<td>pneumonia in immuno-compromised patients</td>
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<tr>
<td>Mycobacterium tuberculosis</td>
<td>tuberculosis</td>
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</table>
Overview of Bacterial infections

Bacterial meningitis
- Streptococcus pneumoniae
- Neisseria meningitidis
- Haemophilus influenzae
- Streptococcus agalactiae
- Listeria monocytogenes

Otitis media
- Streptococcus pneumoniae

Pneumonia
Community-acquired:
- Streptococcus pneumoniae
- Haemophilus influenzae
- Staphylococcus aureus
Atypical:
- Mycoplasma pneumoniae
- Chlamydia pneumoniae
- Legionella pneumophila

Tuberculosis
- Mycobacterium tuberculosis

Skin infections
- Staphylococcus aureus
- Streptococcus pyogenes
- Pseudomonas aeruginosa

Eye infections
- Staphylococcus aureus
- Neisseria gonorrhoeae
- Chlamydia trachomatis

Sinusitis
- Streptococcus pneumoniae
- Haemophilus influenzae

Upper respiratory tract infection
- Streptococcus pyogenes
- Haemophilus influenzae

Gastritis
- Helicobacter pylori

Food poisoning
- Campylobacter jejuni
- Salmonella
- Shigella
- Clostridium
- Staphylococcus aureus
- Escherichia coli

Sexually transmitted diseases
- Chlamydia trachomatis
- Neisseria gonorrhoeae
- Treponema pallidum
- Ureaplasma urealyticum
- Haemophilus ducreyi

Urinary tract infections
- Escherichia coli
- Other Enterobacteriaceae
- Staphylococcus saprophyticus
- Pseudomonas aeruginosa

Medically relevant

gram-positive

- Bacillus - degrades complex macromolecules- dust, water, plants, animals fur
- Bacillus anthracis: Common in cattle, Bioterrorism
- Bacillus cereus: food, rice, potatoes, meat
- Clostridium perfringens: progressive, toxins diffuse to healthy tissue, Surgical, compound fractures, sores, septic abortions, gunshot wounds, crushing injuries with dirt

gram-negative cocci

- sexually transmitted disease (Neisseria gonorrhoeae)
- meningitis (Neisseria meningitidis)
- respiratory symptoms (Moraxella catarrhalis).

gram-negative bacilli

- respiratory problems (Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa)
- urinary problems (Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens)
- gastrointestinal problems (Helicobacter pylori, Salmonella enteritidis, Salmonella typhi
Bacteria under the electron microscope

- **Escherichia Coli**
  - EF6691 5.0 kV x15.0K 2.00μm

- **Staphylococcus Aureus**

- **Cholera**

- **Pseudomomas Aeruginosa**
The mode of action of antibacterial compounds

- Inhibition of metabolism (antimetabolites): Sulfonamides
- Inhibition of bacterial cell wall synthesis: Penicillins, Cephalosporins, Vancomycin
- Interaction with the plasma membrane: Polymyxin, Tyrothricin
- Disruption of protein synthesis: Rifamycins, aminoglycosides, tetracyclines, chloramphenicol
- Inhibition of nucleic acid transcription and replication: Nailidixic acid, proflavine
The introduction of antibacterials

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<thead>
<tr>
<th>Year</th>
<th>Class</th>
<th>Target</th>
<th>Example</th>
<th>Structure</th>
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<td>β-lactam</td>
<td>cell wall</td>
<td>penicillin G</td>
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<td>1952</td>
<td>macrolides</td>
<td>protein biosynthesis</td>
<td>erythromycin A</td>
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<td>1958</td>
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<td>Year</td>
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<td><strong>β-Lactams</strong></td>
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<td>dicloxacillin, nafcillin, ampicillin, amoxicillin, carbenicillin,</td>
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<td>Cephalosporins</td>
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<td><strong>Others</strong></td>
<td>Metronidazole, polymyxin, trimethoprim</td>
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</table>
History of Antimetabolites

Paul Ehrlich developed staining dyes, and for example discovered mast cells. He tested more than 100 synthetic dyes for biological activity against *Trypanosoma equinum*, responsible for a disease that horses suffered from.

The first active compound was **trepan red**

```
NaO(S-N-N-S-O-)
```

trepan red

**trepan red** belongs to the class of **azo dyes**

```
CH3-N=N-N=CH3
```

methyl-orange

**salvarsan** is an anti-syphilis compound that is chemically related


Hoechst was a company that produced ago dyes, and the discovery of these antibiotics triggered the transition to pharma industry!
Antimetabolites

- **Sulfonamides**

  ![](image)

  - aromatic ring and sulfonamide required, ring must be para-substituted.
  - the sulfonamide nitrogen must be primary or secondary
  - R1 must be H (or acyl), R2 is variable

  ![](image)

  - active form can be formed in vivo (prodrugs)

  1932, Bayer
Antimetabolites

- Inhibit the synthesis of DNA or nucleotide building blocks

Dihydrofolate reductase inhibitors

- Methotrexate

Source of one-C unit for methylations of deoxyuridinemonophosphate (dUMP) to form deoxythymidinemonophosphate (dTMP)

Conversion of FH₄ to Methylen-FH₄ by the serine hydroxymethyltransferase

- Vitamin B₉
- Methotrexate

Methotrexate inhibits the enzyme dihydrofolate reductase (DHFR), which converts folic acid (FH₂) to tetrahydrofolate (FH₄), and further to N⁵,N¹⁰-Methylene-FH₄. This leads to the inhibition of DNA synthesis and cell division in rapidly dividing cells.

Dihydrofolate (FH₂)

Tetrahydrofolate (FH₄)

N⁵,N¹⁰-Methylene-FH₄
Mode of action of sulfonamides

- Sulfonamides **block the biosynthesis of tetrahydrofolate** in bacterial cells. Tetrahydrofolate provides C-1 units for the pyrimide biosynthesis required for DNA synthesis.

- Sulfonamides **mimick p-aminobenzoic acid**, a substrate for dihydropteroate synthetase.

![Chemical Reaction Diagram](image)
Antimetabolites

Thymidylate synthase inhibitors

5-Fluorouracil acts as an suicide inhibitor
Other Antimetabolites

• Trimethoprim: Inhibits dihydrofolate reductase

Applications
• treatment of urinary tract infections
• eye lotions
• treatment of infections of mucous membranes
• treatment of gut infections
Lipid components of mammalian membranes

- Phosphatidylinositol
  - Glycerol
  - Inositol

- Serine
- Ethanolamine
- Choline
- Inositol

Total charge:
- Serine: -1
- Ethanolamine: 0
- Choline: 0
- Inositol: -1
sphingosine

ceramide

sphingomyelin

cholesterol

linoleic acid
Cell wall of gram-positive bacteria
Cell wall of gram-negative bacteria
The Structure of Lipopolysaccharide (LPS)

Repeating unit

O-antigen

Core

Lipid A

E. C.

D31m4

Scheme of LPS

Gln1

Gln2

Kdo4

Kdo3

FA

MMM1

LML

HM2

HM1

MMM2

LMM

HM1

Gln1

Kd o 3

Kd o 4

MMM1

LMM

HM2

HM1

FA
The peptidoglycan layer

Pentaglycine link inhibited by penicillin

NAM

NAG
Cell Wall Biosynthesis

Growing cell wall

Cross-linking

Vancomycin

Transglycosidation

Gly

NAG

Cell membrane

Bacitracin

Carrier lipid

Cytoplasm

L-Ala

D-Glu

L-Lys

NAM

L-Ala → d-Ala → d-Ala-d-Ala

D-Cycloserine

D-Alanine

Cycloserine

O

H

N

H

NH₂

O

H

Me

H

NH₂

H

HO

C

O

C

O

C

O

C

O
Cell Wall Biosynthesis

• Firstly, N-acetylmuramic acid (NAM) is linked to three amino acids (L-Ala-DGlu-Lys)

• The tripeptide is then linked to D-Ala-D-Ala

• The resulting pentapeptide glycopeptide is attached to a C55 carrier lipid by a translocase enzyme, and carried to the outer surface of the cell membrane

• In the following step N-acetylglucosamine (NAG) is added

• Afterwards a pentaglycine chain is linked to the peptide part

• A transglycosylase enzyme catalyses the attachment of the disaccharide building block to the growing cell wall, and releases the lipid carrier
Chain elongation
Cross-linking

GlcNAc

MurNAc

L-Ala

D-Glu

L-Lys

L-Lys

Gly$_1$ Gly$_2$ Gly$_3$ Gly$_4$ Gly$_5$

FemA

FemB

FemX

TP

V

D-Ala
Discovery of Penicillin

- Alexander Fleming discovers in 1928 that a fungus grew on a bacterial plate containing staphylococci. Close to the fungus all bacteria were killed.
- Biotechnological production of penicillins was established during the second world war and helped saving the life of many soldiers.
- Fleming, Chain und Florey received the Nobel price 1945
Antibacterials that inhibit the cell wall synthesis

- Antibacterial agents which inhibit bacterial cell wall synthesis
- Discovered by Fleming from a fungal colony (1928)
- Shown to be non toxic and antibacterial
- Isolated and purified by Florey and Chain (1938)
- First successful clinical trial (1941)
- Produced by large scale fermentation (1944)
- Structure established by X-Ray crystallography (1945)
- Full synthesis developed by Sheehan (1957)
- Isolation of 6-APA by Beechams (1958-60) - development of semi-synthetic penicillins
- Discovery of clavulanic acid and β-lactamase inhibitors
Penicillin inhibits final crosslinking stage of cell wall synthesis.

It reacts with the transpeptidase enzyme to form an irreversible covalent bond.

Inhibition of transpeptidase leads to a weakened cell wall.

Cells swell due to water entering the cell, then burst (lysis).

Penicillin possibly acts as an analogue of the L-Ala-γ-D-Ala portion of the pentapeptide chain. However, the carboxylate group that is essential to penicillin activity is not present in this portion.

[Chemical structures of Penicillin and Acyl-D-Ala-D-Ala shown]
(a) Transpeptidase cross-linking

(b) Penicillin inhibition
Penicilins

R = Benzyl

R=Phenoxyethyl penicillin (Pen V)

6-Aminopenicillanic acid (6-APA)

β-Lactam ring

Thiazolidine ring

Side chain varies depending on carboxylic acid present in fermentation medium

Penicillin G

present in corn steep liquor

Penicillin V

(first orally active penicillin)
Synthesis of Penicillins

Penicillin G can be enzymatically converted into 6-aminopenicillanic acid (6-APA)

6-APA serves as a convenient starting material for the synthesis of other penicillins
Conclusions

- Amide and carboxylic acid are involved in binding
- Carboxylic acid binds as the carboxylate ion
- Mechanism of action involves the β-lactam ring
- Activity related to β-lactam ring strain (subject to stability factors)
- Bicyclic system increases β-lactam ring strain
- Not much variation in structure is possible
- Variations are limited to the side chain (R)
Resistance to Penicillins

- Gram -ve bacteria have a lipopolysaccharide (LPS) outer membrane preventing access to the cell wall
- Penicillins can only cross via porins in the outer membrane
- Porins only allow small hydrophilic molecules that can exist as zwitterions to cross
- High levels of transpeptidase enzyme may be present
- The transpeptidase enzyme may have a low affinity for penicillins (e.g. PBP 2a for S. aureus)
- Presence of β-lactamases
- Concentration of β-lactamases in periplasmic space
- Mutations
- Transfer of β-lactamases between strains
- Efflux mechanisms pumping penicillin out of periplasmic space
Problems with Penicillin G

- It is sensitive to stomach acids
- It is sensitive to β-lactamases - enzymes which hydrolyse the β-lactam ring
- It has a limited range of activity
Sensitivity to β-Lactamases

- Enzymes that inactivate penicillins by opening β-lactam rings
- Allow bacteria to be resistant to penicillin
- Transferable between bacterial strains (i.e. bacteria can acquire resistance)
- Important w.r.t. Staphylococcus aureus infections in hospitals
- 80% Staph. infections in hospitals were resistant to penicillin and other antibacterial agents by 1960
- Mechanism of action for lactamases is identical to the mechanism of inhibition for the target enzyme
- But product is removed efficiently from the lactamase active site
Sensitivity to $\beta$-Lactamases

- Block access of penicillin to active site of enzyme by introducing bulky groups to the side chain to act as steric shields.
- Size of shield is crucial to inhibit reaction of penicillins with $\beta$-lactamases but not with the target enzyme (transpeptidase).

**Methicillin** (Beechams - 1960)

- Ortho groups important.
CEPHALOSPORINS

7-Aminoadipic side chain

7-Aminocephalosporinic acid (7-ACA)
Properties of Cephalosporin C

Disadvantages
- Polar due to the side chain - difficult to isolate and purify
- Low potency, not absorbed orally

Advantages
- Non toxic
- Lower risk of allergic reactions compared to penicillins
- More stable to acid conditions
- More stable to β-lactamases
- Ratio of activity vs Gram -ve and Gram +ve bacteria is better

SAR
- The β-lactam ring is crucial to the mechanism
- The carboxylic acid at position 4 is important to binding
- The bicyclic system is important in increasing ring strain
- Stereochemistry is important
- The acetoxy substituent is important to the mechanism
- Possible modifications
- 7-Acylamino side chain
- 3-Acetoxyethyl side chain
- Substitution at C-7
Newer β-Lactam Antibiotics

Thienamycin (Merck 1976) (from Streptomyces cattleya)

- Potent and wide range of activity vs Gram +ve and Gram -ve bacteria
- Active vs. Pseudomonas aeruginosa
- Low toxicity
- High resistance to β-lactamases
- Poor stability in solution (ten times less stable than Pen G)
**β-Lactamase Inhibitors**

**Clavulanic acid** (Beechams 1976) (from Streptomyces clavuligerus)

- Weak, unimportant antibacterial activity
- Powerful irreversible inhibitor of β-lactamases - suicide substrate
- Used as a sentry drug for ampicillin
- Augmentin = ampicillin + clavulanic acid
- Allows less ampicillin per dose and an increased activity spectrum
- Timentin = ticarcillin + clavulanic acid
Glycopeptide antibiotics: Vancomycin

Inhibition of transglycosylation by Vancomycin

- Vancomycin forms tight hydrogen bonds with the $\text{DAla-DAla}$ terminal unit of the pentapeptide, thereby capping the pentapeptides.
- Vancomycin can form rather stable head-to-tail dimers.
- Due to the large size of Vancomycin it acts as a steric block preventing access from the transglycosylase and transpeptidase enzymes.
Structure of the complex between Vancomycin and a tripeptide

Knox et al., ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, 1990, 1342-1347
Agents that act on the plasma membrane

Ionophores such as **Valinomycin**, **Nigericin**, **Monensin A** and **Lasalocid A** complex ions and transport them in a non-regulated fashion through the membrane disturbing ion concentrations intra- and extracellularly.
Gramicidin A forms channels that allow passing of ions, but also of nucleosides. Another such peptide is Polymyxin B, that binds with high selectivity to the bacterial plasma membrane.

Recently, lipopeptides such as Daptomycin were discovered that disrupt the cell membrane.
The ribosomes

- Ribosomes are the location of protein biosynthesis.
- Ribosomes consist of both proteins and RNA.
- The active site for peptide bond formation consists solely of rRNA.
- It consists of a large (50S) and a small (30S) subunit.
Ribosomes are made of RNA and proteins

Structure of the 30S subunit from *T. thermophilus*

Structure of the 50S subunit from *D. radiodurans*
Interference with protein synthesis by blocking protein translation

Protein biosynthesis at the bacterial ribosome

(video)
mRNA passes through two narrow channels on the 30S subunit to be displayed at the interface decoding site where it interacts with the tRNA anticodon.

Initially the start codon is translocated into the P site on the 30S subunit to interact with the initiator tRNA charged with Met.

The second mRNA codon, in the adjacent A site, accepts the next tRNA. Basepair matching is checked by the decoding site.

If it matches, the aminoacyl end of the tRNA is swung to the peptidyl-transferase centre, on the 50S subunit.

The ribosome then moves along the mRNA to bring the next codon into the aminoacyl (A) site. In addition, the tRNA holding the nascent peptide strand is translocated into the peptidyl (P) site.

At the end the full strand is moved into the E (exit) site, from where it is ejected.

The ribosome employs entropic catalysis by positioning substrates, re-organizing water and providing an electrostatic network to stabilize reaction intermediates.
• the chain of the nascent polypeptide passes through a narrow tunnel on the 50S subunit, which is 100 Å in length, and runs from the peptidyltransferase centre to emerge from the back of the ribosome
• the macrolides bind in the narrow tunnel blocking exit of the nascent chain
• during protein synthesis nucleotides A1492 and A1493 are flipped out of the 16S rRNA helix to interact with the mRNA codon and its cognate tRNA anticodon at the ribosomal A-site
• some aminoglycosides antibiotics bind within the helix and induce a similar but not identical flip-out of A1493 and A1492.
• as a result basepair matching is NOT required leading to the addition of incorrect amino acids to the nascent chain.
Aminoglycosides

- at physiological pH charged -> binding to lipopolysaccharide, phospholipids and permeabilize the membrane
- bind to the 30S ribosomal subunit, thereby preventing the movement along the mRNA, so that the triplet code cannot be read
- don’t bind to human ribosomes
- polar molecules, need to be injected, unable to cross the BBB
Tetracyclines

- bacteriostatic, widely prescribed
- broad-band antibiotic against gram-positive and gram-negative bacteria
- bind to 30S subunit of ribosomes, and thereby prevent aminoacyl-tRNA from binding
- passes through membrane via porins
- inhibits also protein synthesis in mammalian cells, but more effective in bacteria

Chlortetracycllin (Aureomycin) \((R^1 = \text{Cl}, R^2 = \text{Me} = \text{OH}, Y = \text{H})\)
Tetracycline \((R^1 = \text{H}, R^2 = \text{Me} = \text{OH}, Y = \text{H})\)
Doxycycline (Vibramycin) \((R^1 = \text{H}, R^2 = \text{Me} = \text{H}, Y = \text{OH})\)
Demeclocycline \((R^1 = \text{Cl}, R^2 = \text{H}, X = \text{OH}, Y = \text{H})\)
Chloramphenicol

- binds to the 50S subunit of the ribosomes, inhibiting the movement of the ribosomes along the mRNA
- used to cure eye infections and typhoid
- quite toxic
- bacteria that contain the gene for chloramphenicol transferase are resistant
Macrolides

- one of the safest antibiotics
- binds to the 50S subunit of the ribosome, thereby inhibiting translocation
- can be orally taken
- acid sensitive

Oxazolidonones

Before protein synthesis the 30S and the 50S subunits must associate to form the 70S ribosome. This step is inhibited
Antibiotics inhibiting nucleic acid transcription and replication:

**Quinolones and fluoroquinolones**

- **1st generation**
  - Nalidixic acid
  - Enoxacin
  - Ciprofloxacin

- **2nd generation**
  - Grepafloxacin
  - Trovafloxacin
  - Clinafloxacin

- **broad-band antibiotics**
- **inhibit the replication and transcription of bacterial DNA by inhibiting the topoisomerase**
- **used for infections involving the urinary, respiratory and gastrointestinal tracts as well as against infections of skin, bone and joints**
during replication, supercoiling of the DNA is removed by helicases
the created tension is released by topoisomerase IV, that cut break both strands of the DNA and rejoin them
fluoroquinones inhibit topoisomerase IV in gram +ve bacteria with 1000 fold selectivity over the human enzyme
in gram -ve bacteria, the topoisomerase II (DNA gyrase), that reintroduces supercoiling after replication and transcription is inhibited
Aminoacridines

- directly interact with bacterial DNA through intercalation
- thereby prevents replication and transcription (but toxic)

Rifamycins

- binds non-covalently to the bacterial DNA-dependent RNA polymerase
- does not attach to eukaryotic RNA polymerase
- bactericidal
- used to treat tuberculosis and staphylococci infections