Antimicrobial drugs I.

Principles of the antibacterial chemotherapy
Modes of action and interactions

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“Magic Bullet”
- Chemicals with selective toxicity
- together with Sahachiro Hata have arsenic and aniline dyes derivatives sought
  606 derivative, Salvarsan was effective against syphilis
- ORIGIN: Selective Stains
- DRUG: Arsphenamine (1910)
  “606” Salvarsan
Gerhard Domagk

- Drugs are changed in the body

**ORIGIN:** Prontosil
  (Only active *in vivo*)

**DRUG:** Sulfanilamide  (1935)

**NOBEL:** 1939
History

Alexander Fleming

- Microbes make antibiotics

ORIGIN: moldy culture plate

DRUG: Penicillin (1928)

NOBEL: 1945
Penicillin

- 1945 Nobel-price:
  - Fleming
  - Florey
  - Chain
History

Selman Abraham Waksman

- Soil Streptomyces make antibiotics
- comes up with definition of antibiotic
- Streptomycin the first antituberculoticum

ORIGIN: Penicillin development
DRUG: Streptomycin (1943)

NOBEL: 1952
Antimicrobial effect

- Extracorporal
  - Desinfection
  - Sterilization

- Intracorporal
  - Chemotherapeutic agents
  - Antibiotica
Antimicrobial drugs:

Chemotherapeutic agent - drug synthesised chemically

Antibiotic – natural agent synthesised by a bacterial or fungal strain
Selective Toxicity

- Cause greater harm to microorganisms than to host
- Chemotherapeutic index = lowest dose toxic to patient divided by dose typically used for therapy
Chemotherapeutic index = :

$$K_i = \frac{dosis \ tolerata \ maxima}{dosis \ curativa \ minima}$$

The higher the index, the more effective chemotherapeutic agent (DTM/DCM)
Antibiotics can be either

• **Broad Spectrum**
  – Kill a wide range of bacteria e.g. Penicillin

• **Narrow Spectrum**
  – Kill a specific type or group of bacteria e.g. Isoniazid
• **Bacteriostatic**, i.e. those that act primarily by arresting bacterial multiplication,

• **Bactericidal**, i.e. those which act primarily by killing bacteria
• **MIC (minimal inhibitory concentration)**
  $\Rightarrow$ the lowest concentration (highest dilution) of the drug that has an inhibitory (bacteriostatic) effect.

- Tube dilution
- Microdilution
- Agardilution
- E-test

• **MBC (minimal bactericidal concentration)**
  $\Rightarrow$ the lowest concentration (highest dilution) that has a killing (bactericidal) effect
The Ideal Drug*

1. **Selective toxicity**: against target pathogen but not against host
   - $\text{LD}_{50}$ (high) vs. MIC and/or MBC (low)

2. **Bactericidal vs. bacteriostatic**

3. **Favorable pharmacokinetics**: reach target site in body with effective concentration

4. **Spectrum of activity**: broad vs. narrow

5. **Lack of “side effects”**
   - Therapeutic index: effective to toxic dose ratio

6. **Little resistance development**
   * There is no perfect drug.
Side effects of chemotherapy

- Allergic response
- Toxic effects
- Disbacteriosi
- Inhibition of immune system
- Embryotoxic action
- Formation of the drug resistance
Types of combined actions of antimicrobial
Antibiotic Mechanisms of Action

**Cell wall synthesis**
- Beta-lactams
- Vancomycin
- Isoniazid
- Ethambutol
- Cycloserine
- Ethionamide
- Bacitracin
- Polymyxin

**DNA replication**
- Quinolones
- Metronidazole

**RNA synthesis**
- Rifampin
- Rifabutin

**DNA**
- mRNA

**Ribosomes**
- 50S
- 30S

**Translation**
- Protein synthesis
  - (50S ribosomes)
  - Chloramphenicol
  - Macrolides
  - Clindamycin
  - Streptogramins

**Translation**
- Protein synthesis
  - (30S ribosomes)
  - Aminoglycosides
  - Tetracyclines
  - Oxazolidinone

**Alteration of Cell Membrane**
- Polymyxins
- Bacitracin
- Neomycin

**Antimetabolites**
- Sulfonamides
- Dapsone
- Trimethoprim
- Para-aminosalicylic acid
Beta-lactam antibiotics
Their structure contains a beta-lactam ring. *The major subdivisions are:*

(a) **penicillins** whose official names usually include or end in “cillin”

(b) **cephalosporins** which are recognized by the inclusion of “cef” or “ceph” in their official names.

(c) **carbapenems** (e.g. meropenem, imipenem)

(d) **monobactams** (e.g. aztreonam)

(e) **beta-lactamase inhibitors** (e.g. clavulanic acid, sulbactam).
Beta-lactams effect on bacterial cell wall
Subunits for cell wall construction

- N-acetylmuramic acid
- N-acetylglucosamine
- pentapeptide
- D-ala-D-ala
Cell Wall Assembly

Layer of cell wall with cross links of 5 glycines (gray)

Second layer of cell wall cross-linked to the lower layer

A subunit is added to the growing chain

Transpeptidase (PBP) forms a 5-glycine bridge between peptides
Transpeptidase, or PBP (orange sunburst) is bound by beta-lactam antibiotic (light blue) and its activity is inhibited (turns gray)
5-glycine crosslinking bridges cannot form in the presence of a beta-lactam, and the cell wall is deformed and weakened.
Penicillin

rupture

later

Bacterium

Antibiotic

Water enters

Cell deteriorates

Cell is destroyed
Mechanisms of beta-lactam resistance

• Drug-modifying enzymes (beta-lactamases)
  – Gram-positives (e.g., *S. aureus*) excrete the enzyme
  – Gram-negative (e.g., *E. coli*) retain the enzyme in the periplasm

• Overexpression of cell wall synthetic enzymes
  – e.g., vancomycin-intermediate *S. aureus* (VISA)

• Alteration of the PBPs so antibiotic cannot bind
  – e.g., MRSA, *S. pneumoniae*, gonococcus

• Exclusion from the site of cell wall synthesis
  – Porin mutations in the outer membrane of Gram-negative bacteria only (e.g., *Ps. aeruginosa*)
Beta-lactamases (dark orange) bind to the antibiotics (light blue) and cleave the beta-lactam ring.

The antibiotic is no longer able to inhibit the function of PBP (orange sunburst)
Beta-lactamase inhibitors

• **Bind the beta-lactam ring irreversibly**

• **Clavulanic acid**
  – Augmentin (amoxycillin/clavulanic acid)

• **Sulbactam**
  – Unasyn (ampicillin/sulbactam)

• **Tazobactam**
  – Tazocin (piperacillin/tazobactam)
Resistance to β-Lactams – Gram negative
Beta-lactamases

- **Extended-spectrum beta-lactamase ESBL**
  - Hydrolyze: penicillins, cephalosporins
  - NOT hydrolyze: carbapenems, monobactams
  - Beta-lactamase inhibitors: clavulanic acid, sulbactam, tazobactam will inhibit!
  - Gram-negative bacteria

- **Metallo beta-lactamase (MBL)**
  - Hydrolyze: CARBAPENEMS
  - Gram-negative bacteria
Resistance to β-Lactams – Gram positive
Methicillin resistant Staphylococcus aureus

MRSA

• Penicillin-binding proteins (PBPk) - structure modification

• Resistance to ALL BETA-LACTAM ANTIBIOTICS:
  – Penicillins
  – Cephalosporins
  – Carbapenems
  – Even to Beta-lactamase inhibitors
Glycopeptides:
Vancomycin, Teicoplanin
Komplex Effect - Glikopeptides

- Vancomycin, Teicoplanin
  - interfere with Peptidoglicansynthese
  - destroy the cytoplasmic membrane
  - prevent RNA synthesis

- can not go through Gram-negative cell wall
- Only for Gram-positive bacteria
Inhibition of peptidoglycan cross-linking by Beta-Lactams and Vancomycin and mechanisms of resistance.

1. Transpeptidase enzyme binds to D-Ala-D-Ala for cross-linking.
2. Beta-lactam antibiotic binds to transpeptidase inhibiting cross-linking.
3. Vancomycin binds to D-Ala-D-Ala preventing binding of enzyme.
5. Changing terminal D-Ala to D-Lactate prevents vancomycin binding.
Mechanism of vancomycin action

D-alanyl-D-alanine (D-ala-D-ala)
Mechanism of vancomycin resistance

Vancomycin resistant Enterococcus (VRE)

Vancomycin is unable to bind to the D-ala-D-lactate structure.
ANTIBIOTICS AFFECTING CELL MEMBRANES

• Polypeptides
  – Surface active amphipathic agents.
  – Interact strongly with phospholipids and disrupt the structure of cell membranes.

• Daptomycin
  – Depolarizes the cell membrane
Polypeptids

- Disintegration of the outer membrane
- Narrow spectrum only against G negative (except Proteus, Neisseria)
- Bactericidal Antibiotics
- Kidney toxicity
- Intestinal decontamination-per os are not absorbed
- Eye, ear drops, wound infections
- For example: polymyxin B, colistin (im, iv..)
Mechanism of Action
ALTERATION OF CELL MEMBRANES

- binds to lipopolysaccharide on outer cell wall of GNR;
- permeability change in cell envelope;
- leakage of cell content.
Colistin

- Spectrum: aerobic gram-negative rods, including *Acinetobacter, Ps. aeruginosa, Stenotrophomonas*.
- NOT active against: *Burkholderia, Proteus, Serratia, Brucella*, gram-negative anaerobes, gram-positive cocci
- Adverse effects: Neurotoxicity – dizziness, weakness, vertigo, visual changes, confusion, ataxia.
ANTIBIOTICS INHIBITING PROTEIN SYNTHESIS

- Macrolides
- Clindamycin
- Linezolid
- Streptogramins
- Chloramphenicol
- Tetracyclines
- Aminoglycosides
Proteinsynthesis
Indicates new peptide bond forming between aa₆ and aa₅.

P site

A site

tRNA₄ leaving

aa₇-tRNA₇ arriving

5’ mRNA

3’

Codon aa₁

Codon aa₂

Codon aa₃

Codon aa₄

Codon aa₅

Codon aa₆

Codon aa₇

Movement of ribosomes
Procaryotic Ribosome

50S
30S

70S--M.W.2,500,000

Eucaryotic Ribosome

60S
40S

80S--M.W.4,200,000
Antibiotics binding to the 50S ribosomal subunit and inhibiting protein synthesis

• Erythromycin and other macrolides
• Chloramphenicol
• Linezolid
• Streptogramins
Nascent polypeptide chain

MACROLIDES

Transferase site

mRNA template

TRANSLOCATION
Macrolides: Erythromycin, Clarithromycin, Azithromycin

- **Use:**
  - Broad spectrum against gram positives including Staph aureus (MSSA)
  - Good for atypical organism such as Mycoplasma, Chlamydia, Legionella
  - Covers *N. gonorrhea, H. influenzae, Legionella*

- **Caution:**
  - can interact with statin to cause myopathy
  - Can cause Qt prolongation

- **Side effects:**
  - GI upset
Makrolides and lyncosamides

- Prevent the movement of mRNA to the 50S ribosoma unit
- Bacteriostatic
- Little toxicity
- Effective against intracellular living bacteria, anaerobic streptococci, against Campylobacter
  .: eg erythromycin, azithromycin, Clyndamycin

- Resistance to macrolides
  - Encoded on the Kromosomen: Change in ribosoma unit
  - Encoded on plasmids: efflux
Mechanism of action of Chloramphenicol
Chloramphenicol

- Interfere with the attachment of tRNA on the 50S ribosome unit
- Bacteriostatic
- Broad-spectrum
- Systemic we only use against *H. influenzae* meningitis, and in intraocular infections
- Very toxic
  - Destroys the blood cell is being made (Panzytopenia)
  - Gray Syndroma in neonates with liver damage
  - Dysbacteriosis, necrotising ulcerative colitis
Inhibits the formation of 70S Initiation komplex
August 2005 „Molekule of the Month”
For Gram-positive cocci
Nascent polypeptide chain

QUINUPRISTIN (MACROLIDE)

50S

30S

mRNA

template

Transferase site

DALFOPRISTIN

A

P

aa
Quinupristin-Dalfopristin

- Semisynthetic Streptogramine Derivate
- Inhibition of Peptidyltransferase on the 50 S
- Q. cause conformation changes
- D. better binding
- Stronger effect= Baktericid
- Gram-positive cocci  MRSA
Antibiotics binding to the 30S ribosomal subunit and inhibiting protein synthesis

- Aminoglycosides
- Tetracyclines
A

50S

30S

mRNA template

Transferase site

Nascent polypeptide chain

Tetracycline

P

mRNA

template

Tetracycline
Aminoglycosides

- Inhibit the transcription of the 30S ribosome unit
- The active transport of antibiotics need O2. The anaerobic bacteria are genetically resistant to aminoglycosides.
- The first antibiotic was only the streptomycin against Mycobacterium.
  - Netilmicin, tobramycin, amikacin gentamicin we can locally and systematically use also.
  - Neomycin we only use in eye drops.
Aminoglycoside resistance

- Encoded on the chromosome

- The structure of 30S ribosome unit is changed
  - high level resistance in enterococci

- Permeations inhibition by anaerobic metabolism
  - Low level resistance in enterococci

- Combination with cell wall synthesis inhibitors in endocarditis
  - Encoded on plasmids
  - Antibiotics destroy the enzymes inactivation / structural modification by
    - Acetylation
    - Adenylation
    - phosphorylation
Tetracycline

- Prevents the attachment of tRNA on the 30S ribosome unit
- Bacteriostatic
- Broad-spectrum activity
  - Includes aerobic G+ and G-, atypicals [Rickettsia spp, treponema spp, chlamydia spp, and others]
  - Little to no effect on fungi or viruses

- Tigecycline
  - 70% of Hungarian bacteria are resistant
    - efflux pumps
    - Stabilization of ribosome-tRNA
- Tetracycline, Doxycycline, Minocycline
- New derivatives: tigecycline
• Once inside the cell…
  – Bind 30S ribosomal subunit
  – Blocks binding of aminoacyl-tRNA to acceptor site on mRNA-ribosome complex
  – Protein synthesis is inhibited = bacteriostatic effect
Mupirocin

- Prevents the attachment of Izoleucin – tRNS
- produced by Pseudomonas fluorescens
- Active against only staphylococci and streptococci
- Lokale effect against MRSA (Baktroban)
- Topical treatment of impetigo
Antibiotic Mechanisms of Action

**Cell wall synthesis**
- Beta-lactams
- Vancomycin
- Isoniazid
- Ethambutol
- Cycloserine
- Ethionamide
- Bacitracin
- Polymyxin

**DNA replication**
- Quinolones
- Metronidazole

**RNA synthesis**
- Rifampin
- Rifabutin

**Translation**
- Protein synthesis (50S ribosome)
- Chloramphenicol
- Macrolides
- Clindamycin
- Streptogramins

**Antimetabolites**
- Sulfonamides
- Dapsone
- Trimethoprim
- Para-aminosalicylic acid

**Alteration of Cell Membrane**
- Polymyxins
- Bacitracin
- Neomycin
ANTIBIOTICS ACTING AS ANTIMETABOLITES

- Sulfonamides
- Trimethoprim plus sulfamethoxazole
Mechanism of Action

ANTIMETABOLITE ACTION

A

Sulfonamide

(Hydroxymethyl) dihydropteroidine

Sulfonamides Dapsone

B

PABA

p-aminobenzoic acid

PABA

Dihydropteroate synthase

Dihydropteroic acid

Plus alumatic acid

Dihydrofolic acid

Trimethoprim

Dihydrofolate reductase

Tetrahydrofolic acid
Trimethoprim/Sulphamethoxazole

- Good activity against Gr (+) and Gr (-) organisms: MRSA, very active against PCP. Covers *Stenotrophomonas maltophilia*, *Nocardia*, and enteric gram-negative rods.
- Exceptions: *Pseudomonas aeruginosa*, Group A strep, enterococcus, Gr (-) anaerobes.
- Toxicity: GI upset, rash can progress to SJS and TEN, thrombocytopenia, leucopenia, hepatitis; hyperkalemia
- SMX:TMP is a 5:1 ratio, in oral and IV dosage forms.
Results from multiple mechanisms.

- Altered dihydropteroate synthetase.

- Cross-resistance among all sulfonamides.
ANTIBIOTICS AFFECTING NUCLEIC ACID SYNTHESIS.

• Fluoroquinolones

• Metronidazole

• Rifampin
Rifampin

binds to RNA polymerase

✓ active against gram positive cocci
✓ bactericidal for *Mycobacterium*
✓ used for treatment and prevention of meningocococcus
Metronidazole

Mechanism of action of metronidazole on an anaerobic organism

- Ferredoxin reduced
- Short lived intermediates
- RNA
- Protein
- DNA
- Other targets
- Inactive end products

Inactive End Products
Metronidazole

• Mechanism of action:
  – Enters bacteria via cell diffusion
  – Activated via single reduction step by bacteria → forms radicals → reacts with nucleic acid → cell death

• Spectrum of activity:
  – Anaerobic bacteria
  – Microaerophilic bacteria
  – Protozoa

• Resistance:
  – Rare
  – Mechanism: decreased activation (↓ redox reaction) of drug
Quinolones
Quinolones

- Parent drug: nalidixic acid
Classification

- Quinolones (1\textsuperscript{st} generation)
  - Highly protein bound
  - Mostly used in UTIs

- Fluoroquinolones (2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} generation)
  - Modified 1\textsuperscript{st} generation quinolones
  - Not highly protein bound
  - Wide distribution to urine and other tissues; limited CSF penetration.
Mechanism of Action

- **Dual MOA:**
  1. **Inhibition of bacterial DNA Gyrase (Topoisomerase II):**
     1. Formation of quinolone-DNA-Gyrase complex
     2. Induced cleavage of DNA
  2. **Inhibition of bacterial Topoisomerase IV:**
     1. Mechanism poorly understood

Mechanism of DNA Gyrase
Quinolones

• Drugs: norfloxacin, ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin

• Mechanism of action:
  – Inhibit bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV → rapid cell death
  – Post antibiotic effect: lasts 1 to 2 hours, increases with increasing concentration

• Mechanism of resistance:
  – Chromosomal:
    • Alter target enzymes: DNA gyrase and topoisomerase IV
    • Decreased drug penetration: Pseudomonas, E. coli
  – Plasmid: seen in some K. pneumoniae and E. coli
  – Mutations in both target enzymes are needed to produce significant resistance
Antibiotic Resistance Cycle

Increased Antibiotic Use

Limited treatment alternatives
- More antibiotics
- Increased mortality

Increased healthcare resource use

Increased hospitalization
- More antibiotics

Increase in resistant strains

Ineffective empiric therapy
- Increased morbidity
- More antibiotics
Resistance to antimicrobial drugs

• **Natural**
  – Chromosomal

• **Acquired (mutation and genetic recombination)**
  – Plasmid
  – Integron
  – Transpososome
Transferring Resistance Genes

1. Plasmid transfer
   - Plasmid Donor
   - Plasmid
   - Resistance gene
   - Transfer of free DNA

2. Transfer by viral delivery
   - Virus
   - Bacterium Infected by Virus

3. Bacterium Receiving Resistance Genes
Gene Transfer Facilitates the Spread of Drug Resistance

1. Resistant and non-resistant bacteria exist
2. Bacterium multiply by the billions
3. Non-resistant bacteria receive new DNA.
4. Drug resistant bacteria multiply and thrive.

Bacteria that have drug resistant DNA may transfer a copy of these genes to other bacteria. Non-resistant bacteria become resistant. In the presence of drugs, only drug-resistant bacteria survive.
A bunch of bacteria, including a resistant variety...

...get bathed in antibiotics. Most of the normal bacteria die.

The resistant bacteria multiply and become more common.

Eventually, the entire infection evolves into a resistant strain.

(normal bacterium)  (dead bacterium)  (resistant bacterium)
Resistance mechanisms

- Enzymatic degradation (beta-lactamases)
- Permeability changes (outer membrane protein)
- Target modification (Penicillin binding protein)
- Efflux pumps
Thank you for your attention!