ANTIBIOTICS

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Structure of the lecture

• History of antibiotics
• Principles of antibiotic treatment
• Mode of actions of antibiotics
• Resistance to antibiotics
• Determination of antibiotic sensitivity
HISTORY OF ANTIBIOTICS
History of antibiotics - 1

• 19th century:
  – Louis Pasteur & Robert Koch:
    **Bacteria as causative agents & recognized need to control them**
History of antibiotics - 2

• Plant extracts
  – Quinine (against malaria)
  – Ipecacuanha root (emetic, e.g. in dysentery)

• Toxic metals
  – Mercury (against syphilis)
  – Arsenic (Atoxyl, against Trypanosoma)

• Dyes
  – Trypan Blue (Ehrlich)
  – Prontosil (azo-dye, Domagk, 1936)
History of antibiotics - 3
Paul Ehrlich

- started science of chemotherapy
- systematic chemical modifications ("Magic Bullet")
  no. 606 compound = Salvarsan (1910)
- selective toxicity !!
- developed the **Chemotherapeutic Index**

Chemotherapeutic Index = \( \frac{\text{Toxic Concentration}}{\text{Effective Concentration}} \)
Chemotherapeutic index

\[
\frac{\text{DTM}}{\text{DCM}} \quad \text{the larger, the better}
\]

- **DTM** = dosis tolerata maxima (toxic)
- **DCM** = dosis curativa minima (effective)

- wide or narrow application concentration interval
History of antibiotics - 4
Penicillin- the first antibiotic - 1928

- **Alexander Fleming** observed the killing of staphylococci by a fungus (*Penicillium notatum*)
- observed by others - never exploited
- Florey & Chain purified it by freeze-drying (1940) - **Nobel prize 1945**
- **first used in a patient**: 1942
- World War II: penicillin saved 12-15% of lives
Fleming Museum, London
History of antibiotics - 5

- **Selman Waksman - Streptomycin (1943)**
  - active against all **Gram-negatives**
  - **first antibiotic active against** *Mycobacterium tuberculosis*
  - most severe infections were caused by Gram-negatives and *Mycobacterium tuberculosis*
  - extracted from *Streptomyces*
  - 20 other antibiotics, incl. neomycin, actinomycin

Nobel prize 1952
PRINCIPLES OF ANTIBIOTIC TREATMENT
Principals of antibiotic treatment

Antibiotic

Bacterium
- Wide or narrow spectrum
- Bacteriostatic or bactericidal
- Penetration ability

Patient
- Basic disease
- Drug allergy
- Pregnancy, childhood

Gram + / -
Resistance !!!
Types of antibiotic therapy

• **Targeted**
  – based on sensitivity tests

• **Empiric**
  – based on the symptoms and habits
  – knowledge of local epidemiological data

• **Profilactic**
  – e.g. intestinal operation, dentical surgery
Possible side effects

• **Allergy**
  – penicillins!
  – type I hypersensitivity reaction (anaphylaxis)

• **Toxic effect**
  – kidney, liver (alcoholism!), bone marrow
  – impaired hearing
  – bones, teeth (tetracyclin: complex with Ca\(^{2+}\))
  – fluoroquinolones: Achilles-tendon rupture

• **Disbacteriosis**
  = killing of the normal flora
  – e.g. pseudomembranous colitis by *C. difficile*
Possible targets

- **Inhibition of cell-wall synthesis**
  - inhibition of peptidoglycan cross-linking (*beta-lactams*)
  - inhibition of peptidoglycan synthesis (*vancomycin*)

- **Disruption of cell membrane**
  - polymyxins

- **Inhibition of protein synthesis**
  - at 30S ribosomal subunit (*aminoglycosides, tetracyclines*)
  - at 50S ribosomal subunit (*macrolides, chloramphenicol*)

- **Inhibition of nucleic acid**
  - inhibition of folic acid synthesis (*sulphonamides, trimethoprim*)
  - inhibition of DNA gyrase (*fluoroquinolones*)
  - inhibition of RNA synthesis (*rifampin*)

SELECTIVE TOXICITY !!!
I. Inhibition of cell wall synthesis (bactericid)

Cell wall controls osmotic pressure

- Filamentation
- Lysis
I.1. β-lactams

• Inhibit *transpeptidation* of peptidoglycan chains

• **Important questions:**
  - can be given orally? (acid stability)
  - β–lactamase (enzyme-) stability?
  - good against Gram negatives? (*Pseudomonas, Acinetobacter*!)

**Structure of β–lactam ring:**
(very vulnerable!)
I.1.1. Penicillins

- **natural penicillins:** *penicillin G, V*
- **enzyme stable:** *methicillin, oxacillin* (MRSA!!)
- **amino-penicillins:** *ampicillin, amoxicillin* (given *per os*, but not enzyme stable)
- **ureido-penicillins:** *piperacillin, mezlocillin* (nor acid or enzyme stable, but good against *Pseudomonas*)
- **carboxi-penicillins:** *carbenicillin*

**β-lactam ring**

+ 5 membered /=tiazolidin/- ring with sulphur
I.1.2. Cephalosporins

- more possibilities for substitution
- also against Gram negatives!

- I. gen.: cefazolin, cephalexin, ...
- II. gen: cefuroxim, cefaclor, cefoxitin, ...
- III. gen.: cefotaxim, ceftriaxon, ...
- IV. gen.: cefepim, cefpirom
- V. gen.: ceftaroline, ceftobiprol

β–lactam + 6 membered /=cephem-/ ring with sulphur
I.1.3. Carbapenems

- widest spectrum!
- derived from penicillins
- *imipenem*, *meropenem*, *ertapenem*
- class B $\beta$–lactamase = carbapenemase

I.1.4. Carbacephems

- derived from cephalosporins
- *loracarbef*

I.1.5. Monobactams

- *aztreonam*
I.2. Glycopeptides

- **vancomycin**, **teicoplanin**
- giant molecules
- triple effect:
  - cell wall synthesis
  - membrane permeability
  - DNA synthesis (?)
- last resort antibiotics
- **VRE!!**
I.3. Polypeptides

- *Bacitracin*:
  - mainly against *S. aureus* and *Str. pyogenes*, for local treatment (skin infections)
  - by *Bacillus licheniformis*
  - inhibits cell wall synthesis
II. Disruption of cell membrane

• Polymixins (e.g. Colistin):
  – desintegration of cell membrane
  – against Gram-negatives, for local treatment
  – (burns, ear, eye - *Pseudomonas*!)
  – bactericid, narrow spectrum
III. Inhibition of protein synthesis (usually bacteriostatic)

Aminoglycosides, tetracyclines

Macrolides, chloramphenicols
III.1. Aminoglycosides

- **bactericid!**
- act on **30S** ribosomal subunit
- **streptomycin**: also against TB (today: only)
- today mainly:
  - **amikacin, netilmicin**: severe systemic infections
  - **tobramycin, gentamicin**: parenteral or eye drops
  - **neomycin**: eye drops
- **often toxic** (deafness!, kidney failure)
III.2. Tetracyclines

- **chlortetracyclin, doxycyclin, oxytetracyclin (Tetran)**
- act on 30S ribosomal subunit, inhibiting the binding of aminoacil-tRNA
- very wide spectrum (also for animals!)
- active against IC bacteria!!
  - *Chlamydia, Mycoplasma, Rickettsia*
- side effects:
  - liver failure (pregnancy!), kidney failure
  - accumulation in bones (teeth of children!)
  - severe diarrhoea, mucosal inflammation
III.3. Chloramphenicol

• acts on 50S ribosomal subunit
• *Streptomyces venezuelae* (Ehrlich)
• wide spectrum → dysbacteriosisc
• today mainly for:
  – typhus abdominalis, amp\(^R\) *Haem. influenzae*
• but: often in developing countries (cheap)
• *per os*, or eye drops / ointments (Chlorocid)
• toxic effects:
  – bone marrow malfunction
  – „Grey baby syndrome” in newborns
III.4. Macrolides

- act on 50S ribosomal subunit
- inhibit the elongation of peptide chain
- higher concentration: becomes bactericid
- groups:
  - 14 membered ring: *erythromycin, clarithromycin*
  - 15 membered ring: *azythromycin*
  - 16 membered ring: *josamycin*
- wide spectrum (*Streptococci; Bordetella, STD, RTI /Haemophilus, pneumol, Helicobacter, Chlamydia*)
- cross resistance exists!
III.5. Lincosamides

- clindamycin, lincomycin

III.6. Streptogramins

- quinupristin, dalfopristin
- in combination = Synercid

III.7. Ketolids

- telithromycin

III.8. Oxazolidinons

- linezolid
IV. Inhibition of nucleic acid synthesis

IV.1. Quinolons

- inhibition of DNA gyrase (supercoiling)
- original compound: nalidixic acid
- fluoroquinolones (FQs):
  - ciprofloxacin, ofloxacin, norfloxacin, sparfloxacin
- wide spectrum (also IC !)
- newer FQs (wider spectrum, better activity) – mainly against Gram-positive upper RTI:
  - levofloxacin, moxifloxacin, gatifloxacin, gemifloxacin
- Not in pregnancy or for young children!
IV.2. Inhibitors of folate synthesis

- Pteridine
- Para-aminobenzoic acid (PABA)

Dihydropteroic acid synthetase → Dihydropteroic acid

Folic acid → Dihydrofollic acid

Dihydrofollic acid reductase (dhfr) → Tetrahydrofollic acid

DNA synthesis
RNA synthesis
Initiation of Protein synthesis
Nucleotide synthesis
Amino acid synthesis

Sulphamethoxazole = PABA analogue
bacteriostatic

Trimethoprim inhibits dhfr
bactericid

In combination (1:5):
- Sumetrolim
- co-trimoxazole
IV.3. Metronidazol

- against anaerobes + some protozoa
- directly breaks down DNA

\[
\begin{align*}
\text{CH}_2\text{CH}_2\text{OH} & \quad \text{CH}_3 \\
\text{N} & \quad \text{N} \\
\text{O}_2\text{N} & \quad \text{ON} \\
\end{align*}
\]

- activated in the host cells by reduction of the nitro group at low redox potential (anaerobes!)
IV.4. RNA synthesis inhibition

Rifampin

- inhibition of DNA dependent RNA polymerase by binding to its β subunit
- if polymerisation has started already, it is ineffective
- paints tear orange

β subunit
(encoded by rpoB gene)
Aim of combinations

– synergy
  • Sumetrolim: TMP + SMX
  • Synercid: quinupristin + dalfopristin
  • penicillin + gentamycin

– avoiding resistance
  • β-lactam + enzyme inhibitors

– polymicrobial infection

– contraindicated:
  • β-lactam + bacteriostatic !!

Acts only on multiplying bacteria
Inhibits multiplication of bacteria
RESISTANCE TO ANTIBIOTICS
First emergence of resistance

- 1928: discovery of penicillin
- 1940: first identification of a β-lactamase
- 1945: 50% resistance to penicillin in *Staphylococcus aureus*
Antibiotic resistant
*Mycobacterium tuberculosis*

• 1943: discovery of streptomycin

• 21 January 1950: George Orwell died from an untreatable streptomycin-resistant strain of *Mycobacterium tuberculosis*
Natural resistance

- against the antibiotic produced by themselves
- cell wall barrier (Gram-negatives), or lack of cell wall (*Mycoplasma*)
- lack of transport system
- lack of receptors
Acquired resistance - 1

- **vertical**: spontaneous mutations (evolution, selection)

- normal mutation rate: 1 in $10^7$

- selection of resistant mutants:
Acquired resistance - 2

• **horizontal**: giving resistance genes to other bacteria
  
  – by plasmid (conjugation)
  
  – by phage (transduction)
  
  – by transposon (mobile genetic elements)
  
  – by transformation (naked DNS)
Bacterial cell sensitive to ampicillin

Plasmid transfer of antibiotic resistance genes

Bacterial cell resistant to ampicillin

sex pilus

chromosome

R-plasmid
Plasmid transfer of antibiotic resistance genes

Bacterial cell resistant to ampicillin

Bacterial cell RESISTANT to ampicillin
Human reasons leading to resistance

- prescribing antibiotics too often
- too long therapy, too low dose
- stop taking the antibiotic before completing the therapy
- usage of antibiotics in animal husbandry
- spread of resistant hospital strains (hygiene!)

MULTI DRUG RESISTANCE !!!
RESISTANCE MECHANISMS
The 3 major mechanisms

- Penicillins
  - Enzymatic inactivation
  - Altered target

- Sulphonamides

- Tetracyclines
  - Active efflux
1. Enzymatic inactivation

- cleaving (hydrolysis) of antibiotics!!
  - e.g. \( \beta \)-lactamase action on *ampicillin*:
Penicillin + enzyme inhibitor combination

- enzyme inhibitor = β-lactam analogue (suicidal molecules)

- ampicillin-sulbactam = Unasyn
- amoxicillin-clavulanic acid = Augmentin
- piperacillin-tazobactam = Tazocin

penicillin clavulanic acid sulbactam
β-lactamases

• very many different ~
• mostly plasmid-encoded (sometimes chromosomal)
• constitutive or inducible (= in the presence of the β–lactam)

• **ESBL:** extended spectrum β–lactamases !!
  TEM, SHV, CTX, OXA
  by Gram negative bacteria
  
  (*E. coli*, Klebsiella, Pseudomonas, Acinetobacter, …)
1. Enzymatic inactivation - 2

- chemical modification:
  - acetylation
  - adenylation
  - phosphorylation
  - methylation

- aminoglycosides, chloramphenicol

\[
\text{Acetyl CoA} \rightarrow \text{Acetylated Chloramphenicol}
\]

e.g. acetylation of chloramphenicol:
2. Alteration of target by mutation

- decreased or no affinity

- *penicillins (pbp)*,

- *aminoglycosides and macrolides (30S and 50S ribosomal subunits)*,

- *quinolons (gyrase genes: gyrA,B)*
3. Efflux pump

- removal of antibiotic
- not very effective
- *macrolides, quinolons, tetracycline*
4. Overproduction of targets

- e.g. overproduction of PABA (SMX)

5. Metabolite by-pass

- production of another target
  - e.g. an additional dihydrofolate reductase
6. Change of membrane permeability

- blocking active transport
- e.g. MRSA: altered membrane lipid structure
- e.g. tetracycline

7. Decreased modification to active component

- e.g. loss of nitrofurantoin-reductase
Problem bacteria

- *Staphylococcus aureus* – **MRSA, VRSA** (methicillin- and vancomycin resistance)
- *Enterococcus faecalis* and *faecium* – **VRE** (vancomycin resistance)
- MDR, XDR *Mycobacterium tuberculosis*
- Carbapenem resistant Gram negatives
  - *Acinetobacter baumannii*
  - *Pseudomonas aeruginosa* [ESBL]
  - *Klebsiella* spp.
  - *Stenotrophomonas maltophilia*
DETERMINATION OF ANTIBIOTIC SENSITIVITY
Disc diffusion test

• Based on zone diameter:
  – **R** (resistant)
  – **I** (intermediate)
  – **S** (sensitive)

• this is used in routine
• good for screening

“antibiogram”
Determination of MIC

- **MIC = minimal inhibitory concentration**
  - = the minimum concentration (in mg/L) of an antibiotic enough to inhibit the growth of a certain bacterial isolate

- **MBC = minimal bactericid concentration**
MIC determination by diffusion

- **Etest**: concentration-gradient on a strip
MIC determination by dilution

**Agar dilution** (AB mixed into the medium)

**Broth dilution** (AB mixed into the medium)