The Problem of Antibiotic - Resistant and Antibiotic - Tolerant Bacteria

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Deaths per Year by Bacterial Infections at a Hospital (Nosocomial Infections)

Germany: \(\sim \frac{1700}{82\,000\,000} = 0.0020\%\)

EU: \(\sim \frac{25\,000}{700\,000\,000} = 0.0025\%\)

USA: \(\sim \frac{90\,000}{310\,000\,000} = 0.0270\%\)

Estimation:
If no new antibiotics till 2050 \(\rightarrow >10.000.000\) deaths per year world wide
# Development of Chemo-Therapeutic Compounds

<table>
<thead>
<tr>
<th>Year</th>
<th>Inventor/Invention</th>
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<tbody>
<tr>
<td>1863</td>
<td>Antoine Béchamp: Arsenic compounds against Trypanosoma</td>
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<tr>
<td>1906</td>
<td>Paul Ehrlich: Salvarsan against <em>Treponema pallidum</em> (syphilis)</td>
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<td>1930th</td>
<td>Gerhard Domagk: Sulfonamides (Protosil)</td>
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<td>1928</td>
<td>Alexander Fleming: Penicillin</td>
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<td>Production started in 1939</td>
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<td>since 1950 additional antibiotics</td>
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Programme

1. Discovery of penicillin
2. Action of important antibiotics and resistance
3. Persister cells: Toxin-antitoxin systems
4. Mechanisms of transfer of resistance genes
5. Strategies to eliminate antibiotic-resistant and -tolerant bacteria
   4.1 Development of new antibiotics
   4.2 Activation of a species-specific toxin
   4.3 Use of bacterial viruses
   4.4 Use of peptides
   4.5 The perfect antibiotic
5. Summary
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Discovery of Penicillin in 1928

Sir Alexander Fleming
1881 – 1955

Nobel Price of Physiology and Medicine 1945

Bacteria can become resistant!
Penicillium chrysogenum (fungus): Producer of penicillin
E. Chain and Howard Florey: Technical production of penicillin
1941 First treatment of a patient
Lechuguilla Cave in New Mexico

~ 4 million years old bacteria; some resistant against 14 different antibiotics!
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Action of Important Antibiotics

1. Inhibition of DNA replication
2. Inhibition of transcription
3. Inhibition of protein synthesis = translation
4. Inhibition of cell wall synthesis
Resistance Mechanisms

Three different resistance mechanisms have been described:

1. Inactivation or destruction of the antibiotics
2. Altered target proteins
3. Efflux of the antibiotics out of the cell
Inactivation of the Antibiotic, Example 1

**Example**: Beta-lactamases

Cleavage of the ring
Inactivation of the Antibiotic, Example 2

Example: Chloramphenicol-Acetyltransferase

Acetylation of chloramphenicol
Alteration of the Target Molecules by a Mutation

Target molecule = Protein

Protein alteration by a mutation in the corresponding gene coding for the protein

- Arginine – Proline – Tyrosine – Alanine – Lysine -
- Arginine – **Alanine** – Tyrosine – Alanine – Lysine -

Proline: CCA
Alanine: GCA
Tetracycline resistant cells contain a protein located in the inner membrane (TetA) pumping out the antibiotic.
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Discovery of Persister Cells

Observation in 1944 by Joseph Bigger:
1. About $10^9$ *Staphylococcus aureus* cells + penicillin $\rightarrow$ a very few cells survived
2. Growth of these cells to about $10^9$, + penicillin $\rightarrow$ again, only a few cells survived
3. Experiment repeated several times $\rightarrow$ always a few cells survived

**Hypothesis**: A few cells survive since they stop metabolism $\rightarrow$ **persister** cells
Molecular Basis of Persistence

Components:
Toxin and Antitoxin = TA module
Antitoxin neutralizes the toxin
Destroy the antitoxin $\rightarrow$ Toxin acts bacteriostatic or bactericidal

- Degradation or stop of synthesis
- Binding to or destroy of an essential molecule
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Transfer of Antibiotics Resistant Genes to Other Cells

Five different mechanisms have been described:

1. Transformation
2. Transduction
3. Conjugation
4. Fusion of membrane vesicles
5. Nanotubes
Conjugation – Transduction - Transformation

- Conjugation: 1946
- Transduction: 1952
- Transformation: 1944
Fusion of Membrane Vesicles

Membrane vesicles can contain DNA, e.g. plasmids

They can fuse with cells of the same or of different species
Intercellular Nanotubes: 2011

Transfer of proteins, mRNA and DNA
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Development of New Antibiotics = Reserve-Antibiotics

Examples:
1. Carbapenene: Beta-lactame; cell wall synthesis
2. Ciprofloaxacine: Synthetic antibiotic; DNA-synthesis
3. Tigecycline: Derivative of tetracycline
4. Linezolid: Synthetic antibiotic; protein synthesis
5. Tedizolid: Synthetic antibiotic; protein-synthesis
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Addition of a Substance Causing Dissociation of the AT Complex

Addition of the substance:
- Causes dissociation of A and T
- Prevents formation of the AT-complexes
⇒ Toxin causes **killing** (bactericidal) or **stop of growth** (bacteriostatic) of the bacterial cell
To Prevent Formation of the AT-Complex; Antitoxin = RNA

Principle: Antitoxin interacts with the mRNA of the toxin and prevents translation.

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Discovery of Bacterial Viruses (= Bacteriophages = Phages)

Phages have been discovered by two scientists independent of each other:
1. Frederick Twort
2. Félix d’Herelle
The English Bacteriologist Frederick Twort

Discovered phages in 1915

FW Twort (1915) Lancet: 1241-1243
The Canadian Microbiologist Félix d`Herelle

Discovered phages in 1917 as microbes able to kill bacteria

Developed the phage therapy

F d`Herelle (1917) Comptes Rendues Hebdomadaires des Seances de L`academie des Sciences: 373-375
Phage Therapy

Félix d‘Herelle suggested to use phages to kill pathogenic bacteria: Experiments were carried out in Egypt and in Thailand.

1923: George Eliava founded the Eliava Institute in Tbilisi, Georgia.

2018: Different companies worldwide try to establish a phage therapy.
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Use of Small Anti-Bacterial Peptides

Properties:

• Small (6 – 100 amino acids) positively charged amphipathic molecules

• Synthesized by human immune, skin and mucous membrane cells (our immune innate system)
Published Applications

1. **Nisin**: Produced by *Lactococcus lactis*; inhibits growth of many Gram-positive bacteria added to food

2. **D-9-mer**: synthetic peptide; active against MRSA (*Methicilline-Resistant Staphylococcus Aureus*) und *P. aeruginosa*
The Peptide SAAP-148, Part 1

• Peptide LL-37: Immune cells, 37 amino acids
• shortened to 24 amino acids and some of them exchanged

Val-Ala-Leu-Iso-Arg-Ala-Asp-Val-His-
Val-Ala-His-Iso-Arg-Ala-Lys-Val-His-

• Peptide SAAR-148 effective against multiresistant Staphylococci, Enterococci and Pseudomonas

The Peptide SAAP-148, Part 2

- Active against persister cells
- Applications: Infections of the skin, infected burns

**Therapy in the interior of our body:**

To develop specialized active substance **capsules**

First clinical studies started at the beginning of 2018
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The Perfect „Antibiotic“

The perfect „antibiotic“ recognizes three different essential proteins.

The probability that the three different genes coding for three different proteins mutate at the same time in a single cell is extremely unlikely.
The Perfect Antibiotic Binds on Three Different Essential Proteins

Gene A  Gene B  Gene C

Protein A  Protein B  Protein C

Mutation rate: $10^{-6} - 10^{-7}$ per gene and generation

Aim: Identification of an identical protein domain in three different essential proteins
Protein Domains

Examples:
Three proteins B, C and D each consist of two domains, and the A domain is identical in all three of them.
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Summary, Part 1

1. Penicillin has been detected in 1928 per chance.

2. The treatment of patients started in 1941 and a few years later, the first penicillin-resistant patients have been described.

3. Penicillin-tolerant cells have been described in 1944 for the first time and called persister cells.

4. The tolerance is based on a toxin-antitoxin system where the antitoxin inhibits the toxin.
5. After removal of the antitoxin, the toxin causes cell death or stop of growth.

6. I discussed five different possibilities to kill antibiotic-resistant and –tolerant cells.

7. The partially artificial peptide SAAP-148 rises hopes. Will additional be created?
Thank you for your attention!

Questions?