**Bacteria**

Unicellular organism, large domain of prokaryotic, roaming Earth for millions of years

Lacks organelles

No mitochondria

Free DNA

Eukaryotic cell

All animal life on earth is dependent on bacteria. Vital in nutrient cycle: fixation of nitrogen from the atmosphere, vitamin B12, vitamin B9... decomposition of dead bodies.  


**Classification of Bacteria: Gram-stain**

Staining to distinguish and classify bacterial species into two large groups, Gram-positive and Gram-negative.  


**Brief history of antibiotics**

"antimicrobials" in the preantibiotic - millenial traditional medicine


350-550CE

1676  Antonie van Leeuwenhoek - First observation of bacteria

1828  Christian Gottfried Ehrenberg introduced the word "bacterium"

1859  Louis Pasteur - debunked spontaneous generation.

1890  Robert Koch - causative relationship between a microbe and a disease. Nobel Prize in Physiology or Medicine 1905.


1928  Alexander Fleming - "discovers" bacteria growth inhibition around a contaminant mold. First isolation of "penicillin". Nobel Prize in Physiology or Medicine 1945


1940  Mass production of penicillin

1950-70  Golden years of antibiotic discovery

1970-  No new classes of antibiotic, only modifications of existing.

Antibiotic resistance

Issue suggested in 1940, but early studies in 1953 concluded:

“Syphilis has now been treated with arsenicals for about 40 years without any indications of an increased incidence of arsenic-resistant infections, and this work gives grounds for hoping that the widespread use of penicillin will equally not result in an increasing incidence of infections resistant to penicillin”.


A growing number of infections, such as pneumonia, tuberculosis, gonorrhoea, etc., are becoming harder to treat as the antibiotics become less effective.

Antibiotic resistance:
- Is one of the biggest threats to global health, food security, and development today.
- Can affect anyone, of any age, in any country.
- Occurs naturally, but misuse of antibiotics is accelerating the process.
- Leads to longer hospital stays, higher medical costs and increased mortality.

1.3 millions Cancer deaths 2015
25792 Traffic deaths 2016
25000 Antibiotic-resistant deaths 2009
33000 Antibiotic-resistant deaths 2018

32% increase in 10 years


Extra healthcare costs and productivity losses
1.5 billion €
Max-Planck-Gesellschaft public budgeted 2017
1.7 billion €
Renault-Nissan net benefits 2018
3.3 billion €

Basic resistance mechanisms

- Intrinsic resistance
  Phenotine of each bacteria makes them +/- sensitives to each antibiotic

- Decreased permeability & Efflux Pumps
  Alterations of pore proteins & Production of pumps to extrude a toxic compound

Chemical alterations of the antibiotic
- Protection of enzymes or modification of the target
- Also complete replacement enzyme

Resistance obtainment

Mutation
- Remarkable genetic plasticity
- High replication rates
- Numerous healthy muted descendants

Horizontal Gene Transfer
- Acquisition of foreign DNA (plasmid)
- Bacteria receiving plasmid with resistant gene

How does resistance occur?
1. Normal Gene
2. Mutated Gene
3. Abnormal Protein
4. No Protein

**β-Lactam antibiotic**

Most widely used group of antibiotics.

Until 2003, more than half of antibiotics sales.

1928 - Fleming found a plate with *Staphylococci* was contaminated by mould with a halo of inhibited bacterial growth.
1942 - 3 month Merck production was available for only 10 patients
1944 - Improved production to 2.3 million doses in time for the invasion of Normandy
1945 - Available for civil use

Biosynthesis of penicillin:
1) Condensation of three amino acids: L-a-aminoadipic acid, L-cysteine, L-valine into a tripeptide and epimerization to D-Valine.
2) Oxidative cyclisation to isopenicillin.
3) Transamidation

### β-Lactam action

β-Lactams stop the synthesis of the peptidoglycan of bacterial cell walls, by the enzyme that cross-branches the peptides.

### β-Lactam resistance

Destruction by β-lactamases

**β-Lactamase inhibitor** can help to overcome antibiotic resistance


Within the tug-of-war of antibiotics and bacteria, more elaborate β-lactams are needed.

**WHO essential medicines**

- Cefotaxime 1980 - 3rd Gen cephalosporin
- Ceftriaxone 2010 - 5th Gen cephalosporin
**Glycopeptides - Vancomycin**

Branched complex tricyclic glycopeptide of produced by *Amycolatopsis orientalis* bacteria.

Serious and life-threatening infections by Gram-positive bacteria unresponsive to other antibiotics.

First glycopeptide on sale in 1954.

WHO Essential medicines.


Vancomycin for use is prepared exclusively by biosynthesis

Some total synthesis e.g. Evans and Nicolaou. Multiple paper synthesis.

For instance, quick search of Nicolaou's synthesis = at least 7 papers for fragments, new methodologies, endgame and sugar attachments.

**Glycopeptide action**

Inhibition cell wall synthesis bind to the terminal moiety of the peptidoglycan precursors preventing cross-linking.

**Glycopeptide resistance**

Acquisition of genes that remodels the synthesis of peptidoglycan by changing the terminal D-Ala for either D-lactate or D-serine to prevent them binding to the precursors.

D-lactate binding glycopeptides.

Gram-negative bacteria are intrinsically resistant to glycopeptides due to various factors related to entering the outer membrane.

*Microbiol Spectr. 2016, 4, 1.*

**Polymyxins**

Cyclic polypeptides produced in some Gram-positive bacteria such as *Paenibacillus polymyxa*.

Colistin, first on sale, was widely discontinued because of toxicity.

Emergency solution for multi-drug resistant infectons.

Polymyxins for use is prepared exclusively by biosynthesis.

Preparation of polymyxins in laboratory are carried out via solid phase syntheses. This way, more active analogs have been prepared.

*Rev Anti Infect Agen, 2005, 40.*

*Journal of Antimicrobial Chemotherapy, 2018, 73, 3385; Special issue in Molecules soon.*

**Polymyxin action**

Bind to lipopolysaccharides in the outer membrane of Gram-negative bacteria, allowing the hydrophobic chain to disrupt cell membranes forming pores where the cellular content leak.

*Communications Biology, 2019, 2, 67.*

Relative absence of bacterial selectivity, since all cells have lipopolysaccharides, explaining toxicity.

Still, WHO Essential Medicines


**Polymyxin resistance**

Changes in the expression of lipopolysaccharides reducing binding of the drug.

Gram-positive bacteria are intrinsically resistant to polymyxins due to the thick layer of peptidoglycan.
**Tetracyclines**

WHO essential medicine extensively used in the prevention and therapy of human and animal infections.

Tetracycline was found in ancient human remains.


Compounds produced from several species of *Streptomyces* bacteria or produced semi-synthetically.


Still, there are some total synthesis, such as (-)-doxycycline 1966 by Myers (2005).

**Aminoglycosides**

Natural or semi-synthetic amino sugars.

Streptomycin was isolated in 1943 from *Streptomyces griseus* & first antibiotic cure for tuberculosis. WHO essential medicines.

Waksman won Nobel Prize in Physiology or Medicine 1952.

*Biochemistry* polyketides and vitamins. 2000, Berlin: Springer. 52.

**Macrolides**

Polyketide with large macro-cyclic lactone ring (14-, 15-, or 16-) and one or more deoxy sugars.

Erythromycin, a 14-member lactone produced by *Saccharopolyspora erythraea* bacteria, first macrolide released to the market in 1952. WHO essential medicines.

*Biochemistry* polyketides and vitamins. 2000, Berlin: Springer. 52.

Though erythromycin A is exclusively produced by biosynthesis, the first total synthesis accomplished by Woodward in 1981 remains a milestone in organic chemistry.


**Action & Resistance**

Tetracyclines, aminoglycosides and macrolides are bacteriostatic agent, stop bacteria from reproducing (not necessarily kill).

Inhibiting the protein synthesis binding to the ribosomes.

Resistance to those type of molecules arise from:

- Decreased permeability / increased efflux.
- Modification of the antibacterial molecule / protection of the ribosome.
- Intrinsec resistance, e.g. Gram-negative are somehow impermeable to macrolides.
**Quinolones**

Synthetic large group of broad-spectrum bacteriocidal agents sharing bicyclic core structure related to 4-quinolone.

Accidental discovery in 1962 of nalidixic acid. Development of a library of quinolone compounds.

_Note: Clinical Infectious Diseases, 2005, 41, S113._

Nearly all quinolones in use are fluoroquinolones.

Effective against both Gram-negative and Gram-positive bacteria.

Ciprofloxacin, one of the most widely used antibiotics worldwide. WHO essential medicines.

_Journal of Antimicrobial Chemotherapy, 2003, 51 (Suppl. S1), 1._

Second Bayer synthesis of ciprofloxacin:

**Quinolone action**

Bacteria lack organelle and nucleus, they have uncaged supercoiled circular double-stranded piece of DNA.

During DNA replication and transcription, becomes overwound ahead of a replication fork.

Torsion eventually stop the DNA or RNA polymerases involved in these processes to continue down the DNA strand.

Gyrase (and topoisomerase IV) relax supercoils, allowing polymerases continue.

Inhibit selectivity the bacterial gyrase and topoisomerase IV leading to cell death.

_Clinical Science, 2016, 130, 1165._

**Quinolone resistance**

Most commonly prescribed antibiotic to adults in 2002. Nearly half (42%) of them for conditions not approved by the FDA, including for some caused by virus.

Resistance to quinolones can evolve rapidly, even during a course of treatment.

_The American Journal of Medicine, 2008, 118, 259; Clinical Infectious Diseases, 2007, 44, 977._

Three types of resistance mechanisms are identified:

- Efflux pumps to decrease intracellular quinolone concentration.
- Plasmid-mediated genes that produce proteins that can bind to DNA gyrase, protecting it from the action of quinolones.
- Mutations at key sites in gyrase or topoisomerase IV to decrease their binding affinity to quinolones.

**Sulfonamides**

Earliest antibacterials to be used systemically, antibiotic revolution in medicine.

Prontosil 1935 - discovered in Bayer result of five years of testing thousands compounds related to azo dyes as antibacterial drugs in the body.

Effective against some important bacterial infections **only in live mice**. Gerhard Domagk, the director of the tests, Nobel Prize in Medicine 1939.

The drug was metabolized to the active sulfanilamide.

Established the concept of **bioactivation** and **pro-drugs**.

*C. R. Soc. Biol., 1935, 120, 756.*

However, sulfanilamide was widely used in the dye industry, the 1909 patent was expired and the drug was available to anyone to modify.

*Deutsches Reich Patentschrift 226239, 1909.*

In 1937, S. E. Massengill Company marketed "Elixir Sulfanilamide", a preparation of sulfanilamide in diethylene glycol killing ~100 patients.

This led to the 1938 Federal Food, Drug and Cosmetic Act, which required proof of safety before the release of a new drug and approvation by the FDA.


Cheap and easy to link to other molecules => thousand of second-generation sulfonamide arised.

Nowadays their use is residual. Only one sulfonamide on WHO essential medicine.

**Sulfonamide action**

Competitive inhibitors of dihydropteroate synthase, stoping the folic acid (vitamin B9) production.

Folate is essential to make DNA, RNA, and metabolise amino acids, which are required for cell division. Bacteriostatic.

Selectivity - mamals are unable to synthesize folate, it is required from the diet, making it an essential vitamin (WHO essential medicine list too)

**Sulfonamide resistance**

Commonly based on pathogen's capability to use other external folic acid precursors or sources.


In general, there is a fitness cost when a bacteria gains resistance to a molecule.

Reduction in clinical sulphonamide usage => constant prevalence of sulfonamide resistance.

Sulfonamide resistance is not harmful for bacteria => won't disappear in short term.