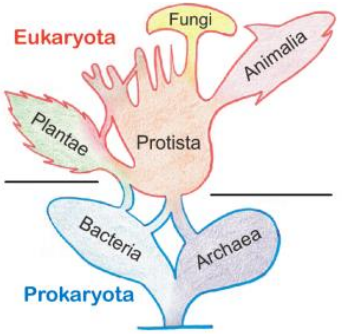
Alejandro Gómez

Antibiotics

Cornella Group Meeting
12.07.2019

Bacteria

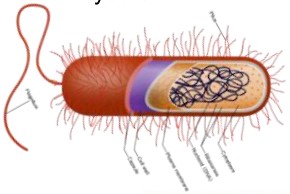


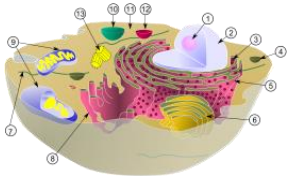
Unicellular organism, large domain of **prokaryotic**, roaming Earth for millions of years

Lacks organelles

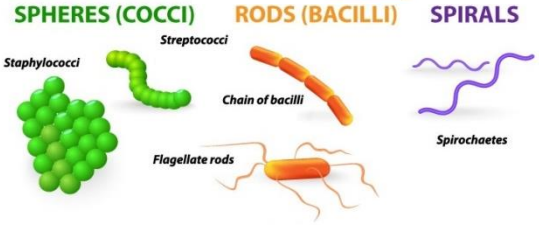
No mitochondria

Free DNA





Eukariotic cell



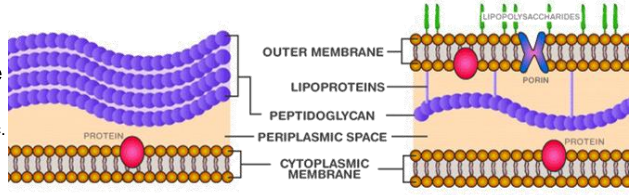
All animal life on earth is dependent on bacteria. Vital in nutrient cycle: fixation of nitrogen from the atmosphere, vitamin B12, vitamin B9... decomposition of dead bodies.

Biochemical Society Transactions, **2012**, *40*, 581.

Classification of Bacteria: Gram-stain

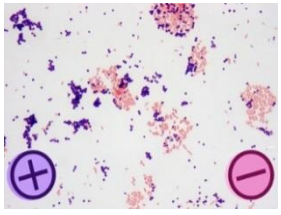
Staining to distinguish and classify bacterial species into two large groups, Gram-positive and Gram-negative. Gram Staining. Current Protocols in Microbiology, **2005**, 00: A.3C.1-A.3C.2.

Gram-Positive
Staphylococcus, Streptococcus, etc.

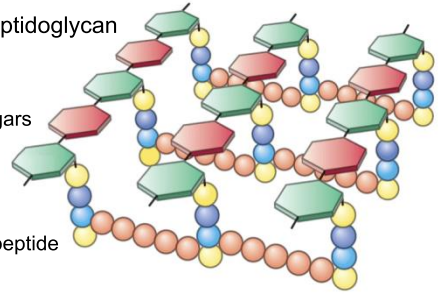


Gram-Negative
Escherichia, Salmonella, etc.

Stained mixture of bacteria



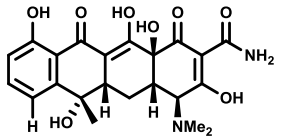
Peptidoglycan



Brief history of antibiotics

"antimicrobials" in the preantibiotic - millennial traditional medicine

350-550CE The traces of tetracycline in human skeletal remains from ancient Sudanese Nubia & Dakhleh Oasis, Egypt. Science **1980**, *209*, 1532; Am. J. Phys. Anthropol. **2010**, *143*, 151; Am. J. Phys. Anthropol. **1989**, *80*, 137.



tetracycline, Ca quelating

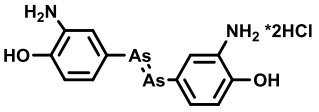
1676 Antonie van Leeuwenhoek - First observation of bacteria

1828 Christian Gottfried Ehrenberg introduced the word "bacterium"

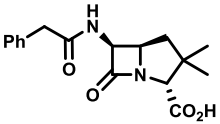
1859 Louis Pasteur - debunked spontaneous generation.

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

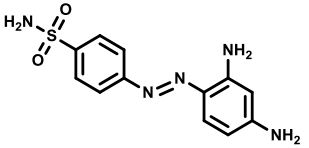
1910 Paul Ehrlich - salvarsan, the first synthetic antibiotic for syphilis. Nobel Prize in Physiology or Medicine 1908. Die Experimentelle Chemotherapie der Spirilosen. Berlin: Julius Springer.



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



1935 Sulfonamide antibiotics - Prontosil, first massively used antibiotic WWII. Nobel Prize in Physiology or Medicine 1939. Dtsch. Med. Wochenschr, **1935**, *61*, 250.



1940 Mass production of pennicilin

1950-70 Golden years of antibiotic discovery

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

today Antibiotic resistance golden era. *The Journal of antibiotics,* **2017**, *70*, 3

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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"[2]

WRONG

Nature, **1940**, *146*, 837; [2] *Br. J. Pharmacol. Chemother.* **1952**, *7*, 33.

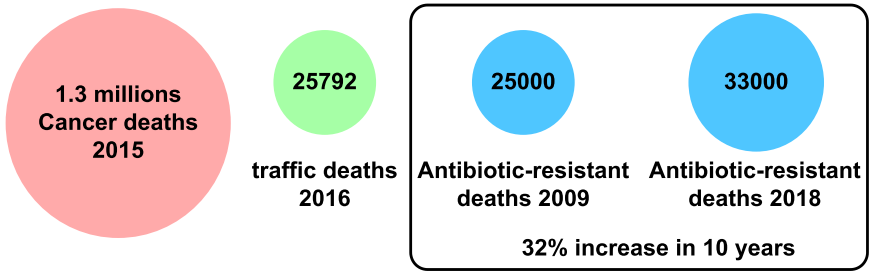
A growing number of infections, such as pneumonia, tuberculosis, gonorrhoea, etc, are becoming harder to treat as the antibiotics become less effective.

World Health Organization

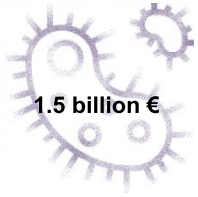
ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

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.



ECDC/ EMEA Joint Working Group, **2009**;https://ecdc.europa.eu/en; https://ec.europa.eu/eurostat

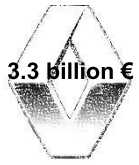


1.5 billion €

extra healthcare costs and productivity losses

1.7 billion €

Max-Planck-Gesellschaft public budged 2017



3.3 billion €

Renault-Nissan net benefits 2018

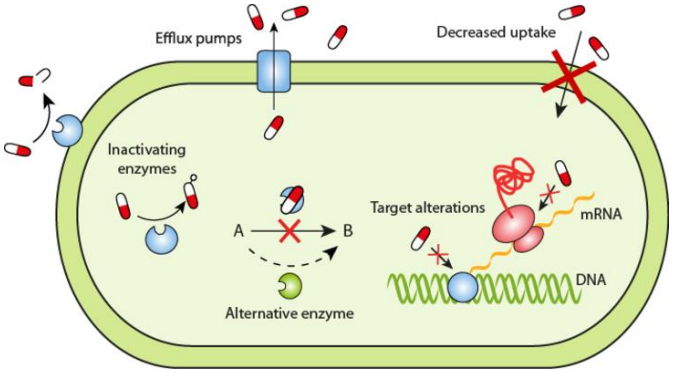
Basic resistance mechanisms

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

Protuction of enzymes to **modify or destroy the antibiotic**

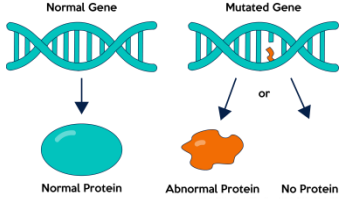
Changes in target sites

Protection or modification of the target
Also complete replacement enzyme

Resistance obtention

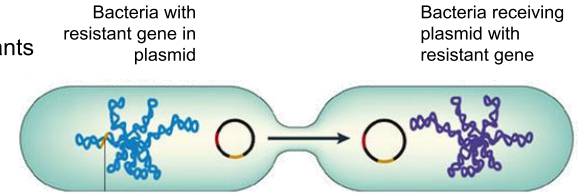
Mutation

Remarkable genetic plasticity
High replication rates
Numerous healthy muted descendants

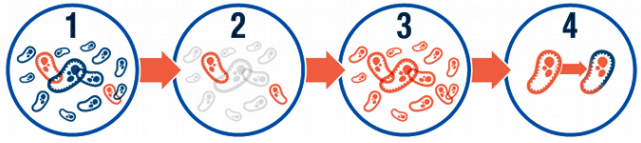


Horizontal Gene Transfer

Acquisition of foreign DNA (plasmid)



How does resistance occur?



Nature Reviews Microbiology, **2015**, *13*, 42; *Microbiol Spectr.* ***2016***, *4*, 1; *Future Sci.* **2018,** *4*, 4.

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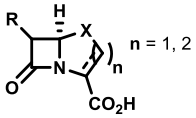
ß-Lactam antibiotic



Most widely used group of antibiotics.

Until 2003, more than half of antibiotics sales.

Applied Microbiology and Biotechnology, **2003**, *61*, 385.

Penicillin, the first ß-Lactam antibiotic:

1928 - Flemming found a plate with *Staphylococci* was contaminated by mould with a halo of inhibited bacterial growth.

1942 - 3 month Merck production was availble for only 10 patients

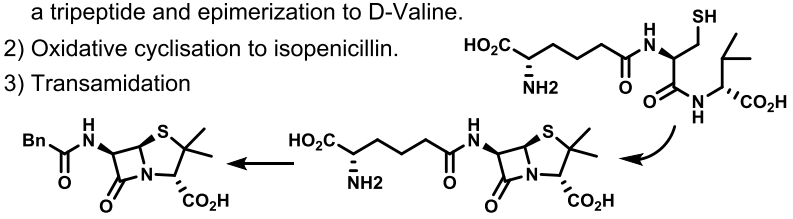
1944 - Improved production to 2.3 million doses in time for the invasion of Normandy

1945 - Available for civil use

Antibiotics **1983**, *History of ß-Lactam Antibiotics* 1-14.

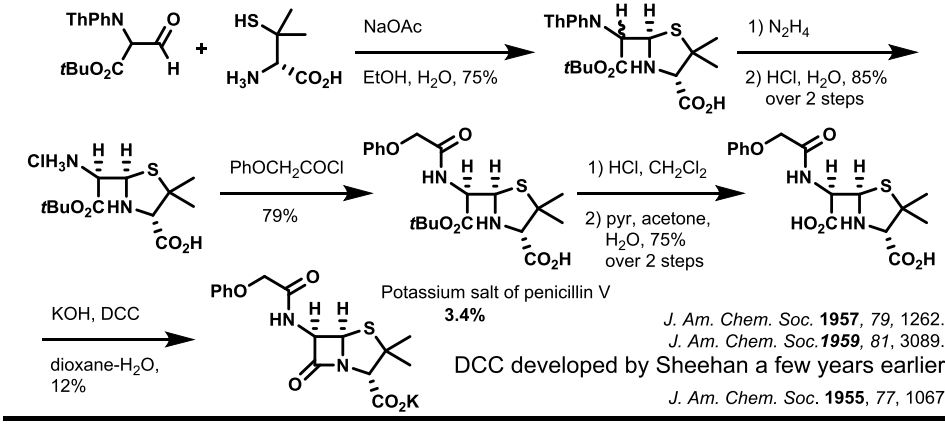
Biosynthesis of penicillin:

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



Advances in Biochemical Engineering/Biotechnology, **2004**, *88*. 45.

Total Synthesis of Penicillin V by Sheehan, 1957

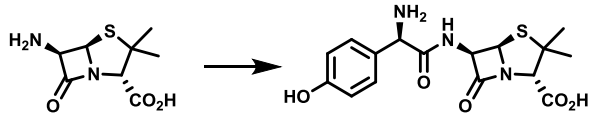


J. Am. Chem. Soc. **1957**, *79*, 1262.
J. Am. Chem. Soc. ***1959***, *81*, 3089.

DCC developed by Sheehan a few years earlier

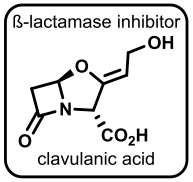
J. Am. Chem. Soc. **1955**, *77*, 1067.

Semisynthetic penicillines and are prepared via modification of intermediates from fermentation of *fungus* molds. *Synthesis of ß-lactam antibiotics*. 2001. Springer. p. 17.



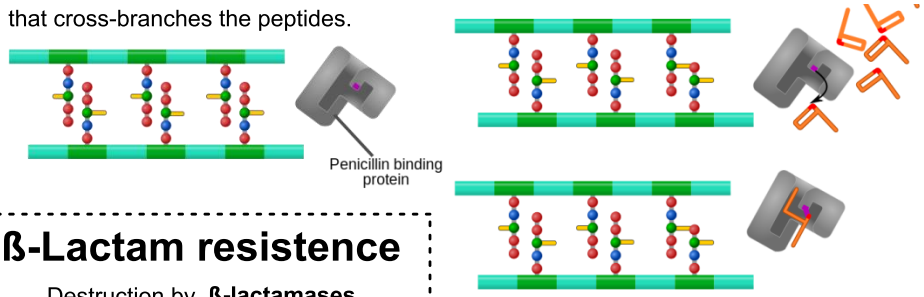
6-APA (+)-6-aminopenicillanic acid

amoxicilin 1972



ß-Lactam action

ß-Lactams stops the synthesis of the peptidoglycan of bacterial cell walls, by the enzime that cross-branches the peptides.

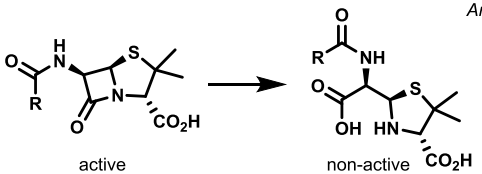


ß-Lactam resistence

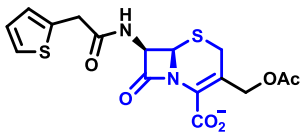
Destruction by **ß-lactamases**

ß-Lactamase inhibitor can help to overcome antibiotic resistance

Nature, **2011**, *477*, 457.
Antimicrobial Agents and Chemotherapy, **1977**, *11*, 852.



More than thousand ß-lactamases have been identified. *Clin. Microbiol. Infect.* **2008**, *14*, 3.

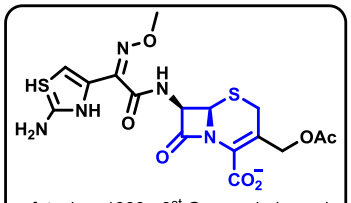


cefalotin 1964 - 1st Gen cephalosporin
& 1st cephalosporin in the market - still used

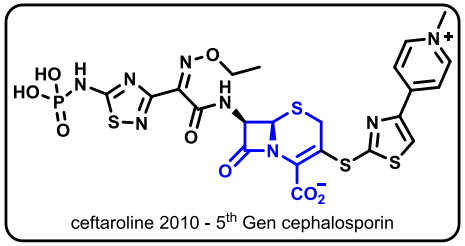
Within the tug-of-war of antibiotics and bacteria, more elaborate ß-lactams are needed.

WHO essential medicines

World Health Organization



cefotaxime 1980 - 3st Gen cephalosporin



ceftaroline 2010 - 5th Gen cephalosporin

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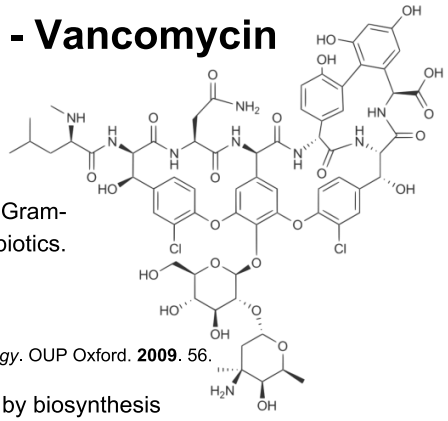
Glycopeptides - Vancomycin



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.

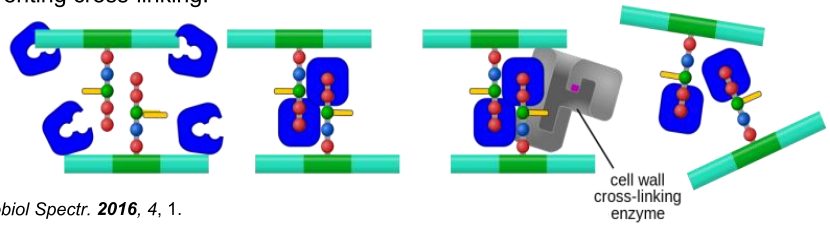
Oxford Handbook of Infectious Diseases and Microbiology. OUP Oxford. **2009**. 56.

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 sythesis = at least 7 papers for fragments, new methodologies, endgame and sugar attachments.

Glycopeptide action

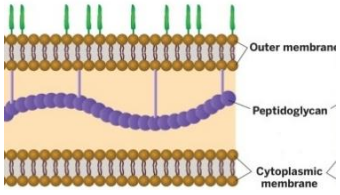
Inhibition cell wall synthesis bind to the terminal moiety of the peptidoglycan precursors preventing cross-linking.



Microbiol Spectr. ***2016****, 4*, 1.

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.

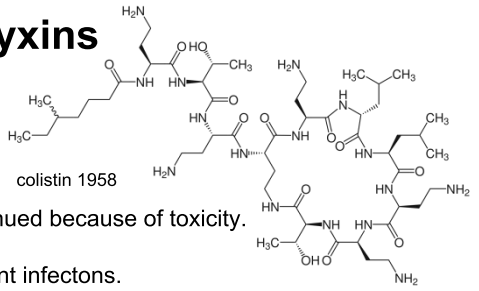


D-lactate binding glycopeptides.

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 1958

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

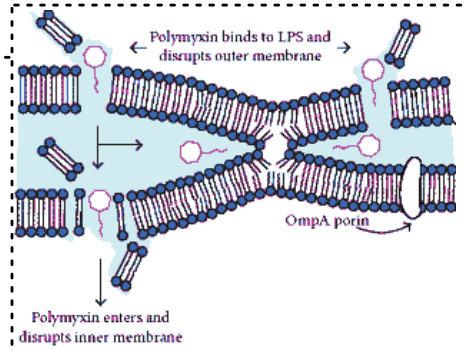
Emergency solution for multi-drug resistant infectons.

Rev Anti Infect Agen, **2005,** *40.*

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.

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.

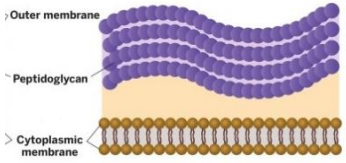
Relative absence of bacterial selectivity, since all cells have lipopolysaccharides, explaining toxicity.

Still, WHO Essential Medicines

Int J Antimicrob Agents. **2005**, *25,* 11

Polymyxin resistance

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



Gram-positive bacteria are intrinsicaly resistant to polymyxins due to the thick layer of peptidoglycan.

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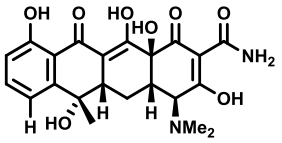
Tetracyclines

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



tetracycline 1955 (chlor 1948)

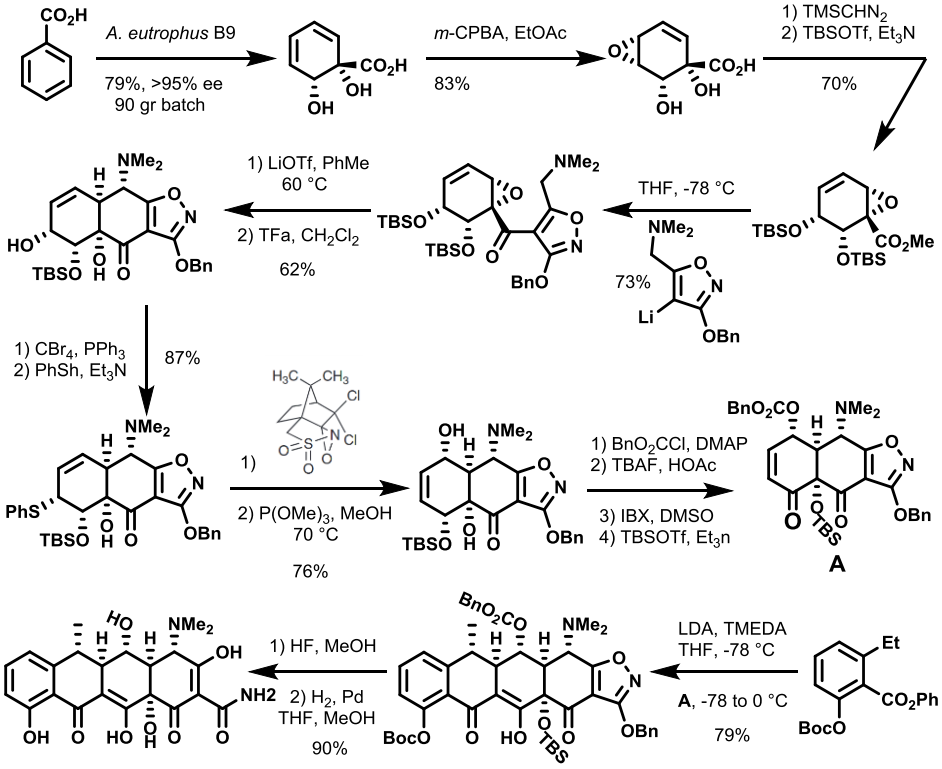
Tetracycline was found in ancient human remains.

Science **1980**, *209*, 1532; Am. J. Phys. Anthropol. **1989**, *80*, 137.

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

Microbiology and Molecular Biology Reviews **2001**, *65*, 232.

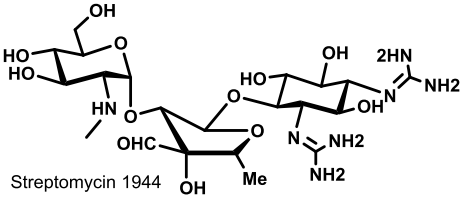
Still, there are some total synthesis, such as (-)-doxycycline 1966 by Myers (2005).



(-)-doxycycline, 18 steps 8.3%

Science, **2005**, *308,* 395.

Aminoglycosides



Streptomycin 1944

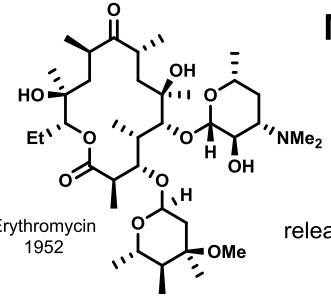
Natural or semi-synthetic amino sugars.

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.

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

Macrolides



Erythromycin 1952

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.

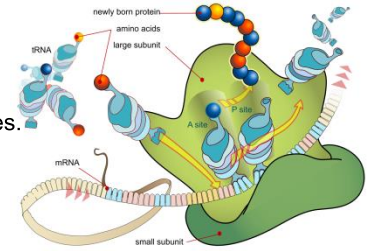
Biosynthesis: polyketides and vitamins. **2000**, *Berlin: Springer.* 52.

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

3 x *J. Am. Chem. Soc.* **1981**, *103, 3210-3213-3215.*

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

Cancer Invest. **1999**,*17,* 87.

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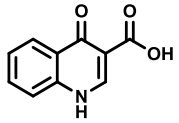
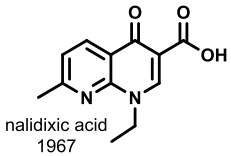
Quinolones

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





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

Clinical Infectious Diseases, **2005**, *41*, S113.

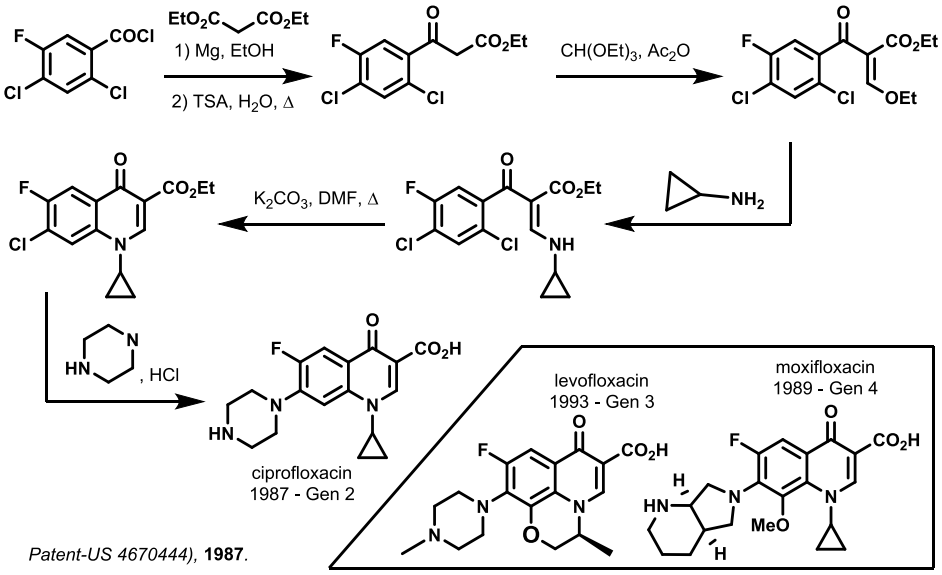
Nearly all quinolone in use are fluoroquinolones.

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.

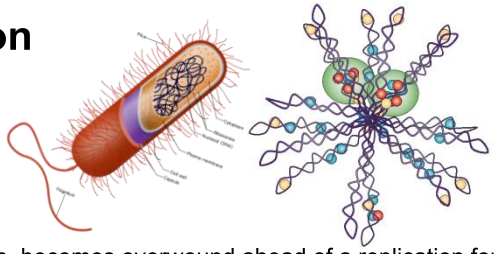
Second Bayer synthesis of ciprofloxacin:



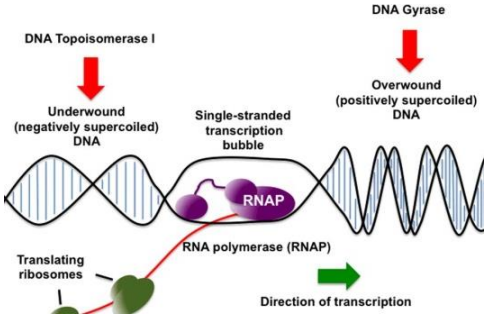
Patent-US 4670444), **1987**.

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 mechanismes 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.

Lancet Infect. Dis. **2006,** *6,* 629; *Antimicrobial Agents and Chemotherapy,* **1998**, *42,* 1778.

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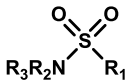
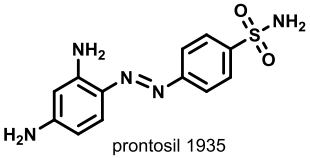


Sulfonamides

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

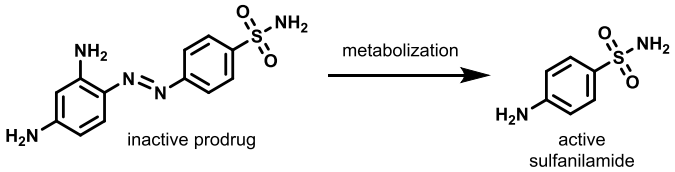
Prontosil 1935 **-** discovered in Bayer result of five years of testing thousands compounds related to azo dyes as antibacterial drugs in the body.


prontosil 1935

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**.

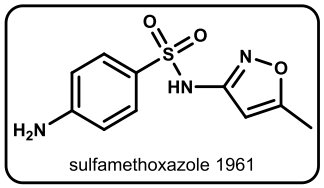
In 1937, S. E. Massengill Company marketed "Elixir Sulfanilamide", a preparation of sulfanilamide in diethylene glycol killing ~100 patients.

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

Ann Intern Med. **1995**,*122,* 456.

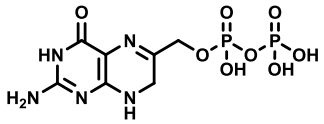
Cheap and easy to link to other molecules => thousand of second-generation sulfonamide arised.

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

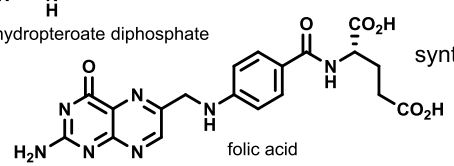

sulfamethoxazole 1961

Sulfonamide action

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


dihydropteroate diphosphate

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


folic acid

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.

Proceedings of the Royal Society of London B, **1997**, *264*, 1287.

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

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

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

Journal of Antimicrobial Chemotherapy, **2004**, *53,* 958.