

Lecture 2: *History of Antibiotics*

Thomas Hermann

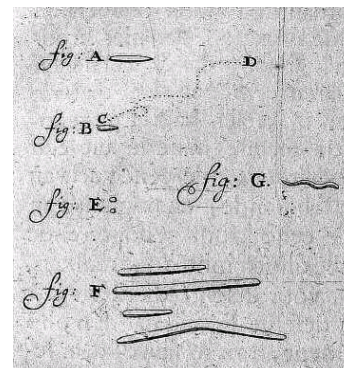
Department of Chemistry & Biochemistry
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Prelude to Antibiotics: Leeuwenhoek & The Birth of Microbiology

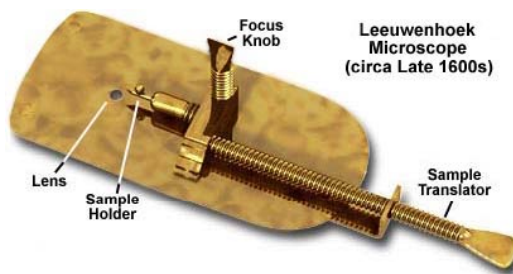


Antonie van Leeuwenhoek
(Delft, 1632-1723)

First to observe and describe single celled organisms which he first referred to as *animalicula*, and which we now know to be microorganisms (*protozoa*, *bacteria*).



Bacteria in tooth plaque (1683)



Leeuwenhoek
Microscope
(circa Late 1600s)



Prelude to Antibiotics: Pasteur, Koch & The Germ Theory of Disease



Louis Pasteur
(Strasbourg, 1822-1895)

Showed that some microorganisms contaminated fermenting beverages and concluded that microorganisms infected animals and humans as well.



Robert Koch
(Berlin, 1843-1910)

Discovered *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Vibrio cholerae* and developed "Koch's Postulates".

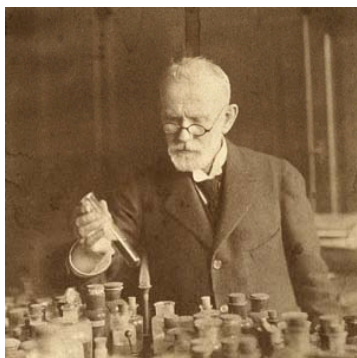
Nobel Prize in Medicine 1905 for work on tuberculosis.

Koch's Postulates: (1890)

To establish that a microorganism is the cause of a disease, it must be:

- 1) found in all cases of the disease.
- 2) isolated from the host and maintained in pure culture.
- 3) capable of producing the original infection, even after several generations in culture.
- 4) recoverable from an experimentally infected host.

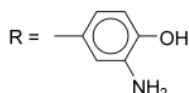
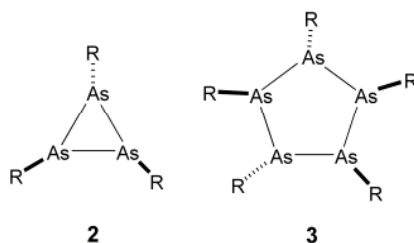
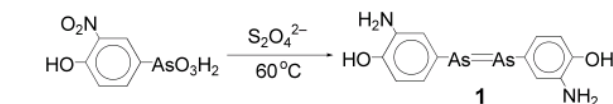
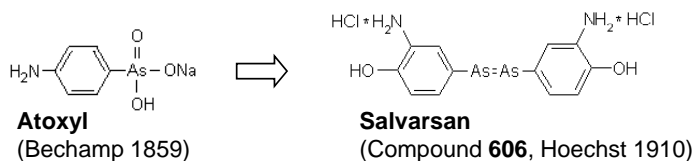
Invention of Modern Drug Discovery: Ehrlich & The Magic Bullet



Paul Ehrlich
(Frankfurt, 1854-1915)

Synthesized and screened hundreds of compounds to eventually discover and develop the first modern chemotherapeutic agent (**Salvarsan**, 1909) for the treatment of syphilis (*Treponema pallidum*).

Nobel Prize in Medicine 1908 for work on immunity (with Mechnikov).

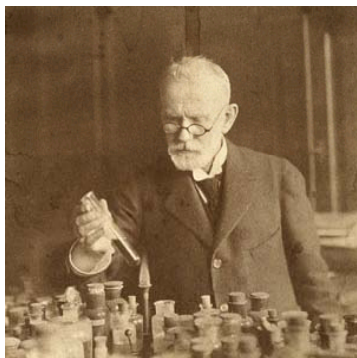


Salvarsan in solution consists of cyclic species (RAs)_n, with *n*=3 (**2**) and *n*=5 (**3**) as the preferred sizes.

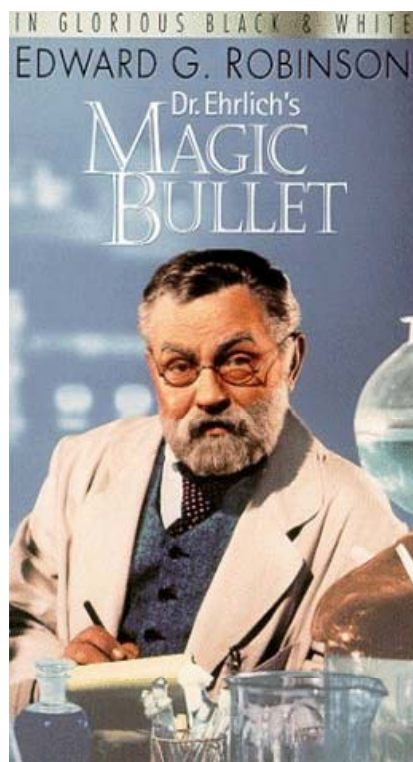
Lloyd et al. (2005) *Angewandte Chemie* 117 (6), 963.

Magic Bullet: Compound that selectively targets a disease causing organism while having no negative effect on human tissue.

Prelude to Modern Drug Discovery: Ehrlich & The Magic Bullet



Paul Ehrlich
(Frankfurt, 1854-1915)



1940 (USA)

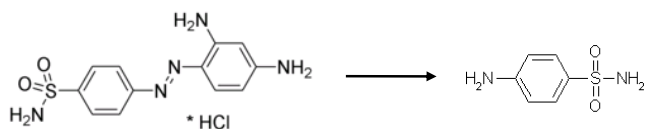
First Antibiotics: Domagk Discovers Sulfonamides ("Sulfa-Drugs")



Gerhard J. P. Domagk
(Wuppertal, 1895-1964)

Worked at Bayer (IG Farben) where he discovered and developed sulfonamides (**Prontosil**), the first drugs effective against bacterial infections.

Nobel Prize in Medicine 1939 for discovery of sulfonamides.



Prontosil (red azo dye)
(Bayer 1935)

Sulfanilamide
(1936)

Prontosil is a prodrug that is not active *in vitro*. Cleavage in the gastrointestinal tract leads to the active compound sulfanilamide which competes with *p*-aminobenzoic acid, the substrate of dihydropteroate synthetase in the bacterial synthetic pathway to folic acid.



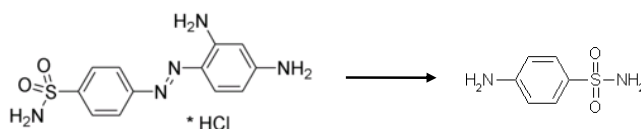
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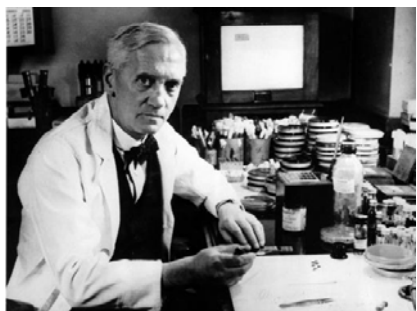
Domagk treated his own daughter with prontosil to fight a severe streptococcal infection and eventually saved her life.

He was forced by the Nazi regime to refuse the Nobel Prize and was finally able to receive the prize in 1947 (but not the monetary portion).

Sulfanilamide had a central role in preventing infection during WW II.

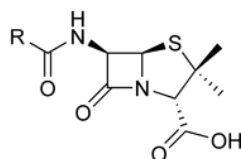
A toxic preparation of sulfanilamide in diethylene glycol (Elixir Sulfanilamide) killed over 100 children in the US, and led to the passage of the Federal Food, Drug, and Cosmetic Act (FD&C) by Congress in 1938 giving authority to the Food and Drug Administration (FDA) to oversee the safety of drugs.

First Antibiotic from a Natural Source: Fleming Discovers Penicillin



Alexander Fleming
(London, 1881-1955)

Nobel Prize in Medicine 1945 for discovery of penicillin (with Chain and Florey).



Penicillin
(R= benzyl, alkyl, etc.)

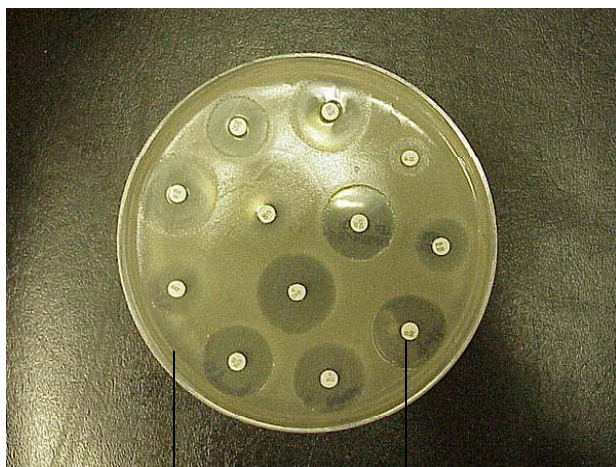


Penicillins are β -lactam antibiotics that act as suicide substrates for peptidoglycan transpeptidases involved in crosslinking the bacterial cell wall.

Alexander Fleming observed, working at St. Mary's Hospital in London in 1928, that a plate culture of *Staphylococcus* had been contaminated by a blue-green mold (*Penicillium*) and that colonies of bacteria adjacent to the mold were being dissolved. The mold grown in pure culture produced a substance that killed bacteria. Naming the substance penicillin, Fleming in 1929 published the results of his investigations (*Brit. J. Exper. Path.* 1929, 10, 226), noting that penicillin might have therapeutic value if it could be produced in quantity (which he was unable to do).

(In 1896, the French medical student Ernest Duchesne originally discovered the antibiotic properties of *Penicillium*, but failed to report a connection between the fungus and a substance that had antibacterial properties, and *Penicillium* was forgotten in the scientific community until Fleming's rediscovery.)

Antibiotic Susceptibility Screening by Disk Diffusion Assay



Agar plate with lawn of bacterial growth.

Paper disks impregnated with antibiotic



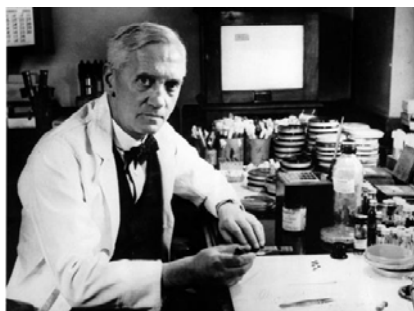
When bacterial multiplication proceeds more rapidly than the drug can diffuse, the bacterial cells that are not inhibited by the antimicrobial will continue to multiply until a lawn of growth is visible and no zone of inhibition appears around the disk.

When the antimicrobial is present in inhibitory concentrations, no growth will appear in the zone around the disk. The more susceptible the bacterial strain tested, the larger the zone of inhibition.

The diameter of the zone of inhibition is indirectly proportional to the minimal inhibitory concentration (MIC).

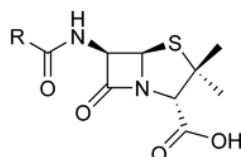
(The MIC assay is readily adapted to liquid cultures. Bacterial growth is measured as optical density at 600 nm, in the absence and presence of different amounts of drug.)

First Antibiotic from a Natural Source: Fleming Discovers Penicillin



Alexander Fleming
(London, 1881-1955)

Nobel Prize in Medicine 1945 for discovery of penicillin (with Chain and Florey).



Penicillin
(R= benzyl, alkyl, etc.)



In 1939, Australian H. W. Florey, German E. B. Chain, and others, working at the University of Oxford, reinvestigated penicillin and demonstrated its *in vivo* efficacy (*Lancet* 1940, 239, 226).

They started to work on the larger scale production of penicillin. In 1941, laboratories at the US Department of Agriculture and Merck & Co joined into penicillin research.

A high yield producing strain (*Penicillium chrysogenum*) was isolated from a moldy cantaloupe that a scientist brought to the laboratory.



Ernst B. Chain
(1806-1979)



Howard W. Florey
(1898-1968)

Within a short period, large scale production of penicillin was established to produce antibiotic for the Allied forces.

First Antibiotic from a Natural Source: Industrial Production of Penicillin



1940: priceless
1943: \$20 /dose
1946: \$0.55 /dose



Photos: Penicillin production in the 1940s



Despite efforts of mass production, availability of penicillin was severely limited. Rapid renal clearance of the drug required frequent administration at high doses. In the early days of penicillin therapy it was common practice to re-isolate the drug from excreted urine of patients for reuse.

First Antibiotic from a Natural Source: Industrial Production of Penicillin



1949 (Great Britain)



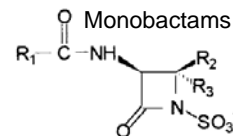
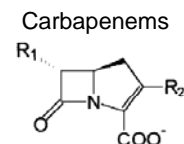
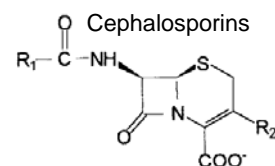
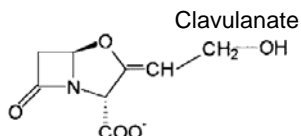
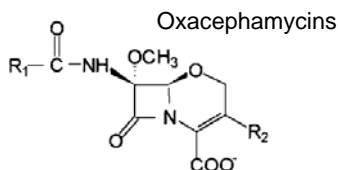
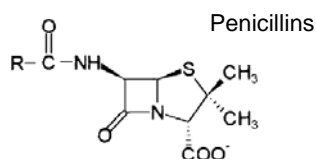
Drug advertising from the 1940s.

First Antibiotic from a Natural Source: Penicillin & β -Lactams



Dorothy M. C. Hodgkin
(Oxford, 1910-1994)

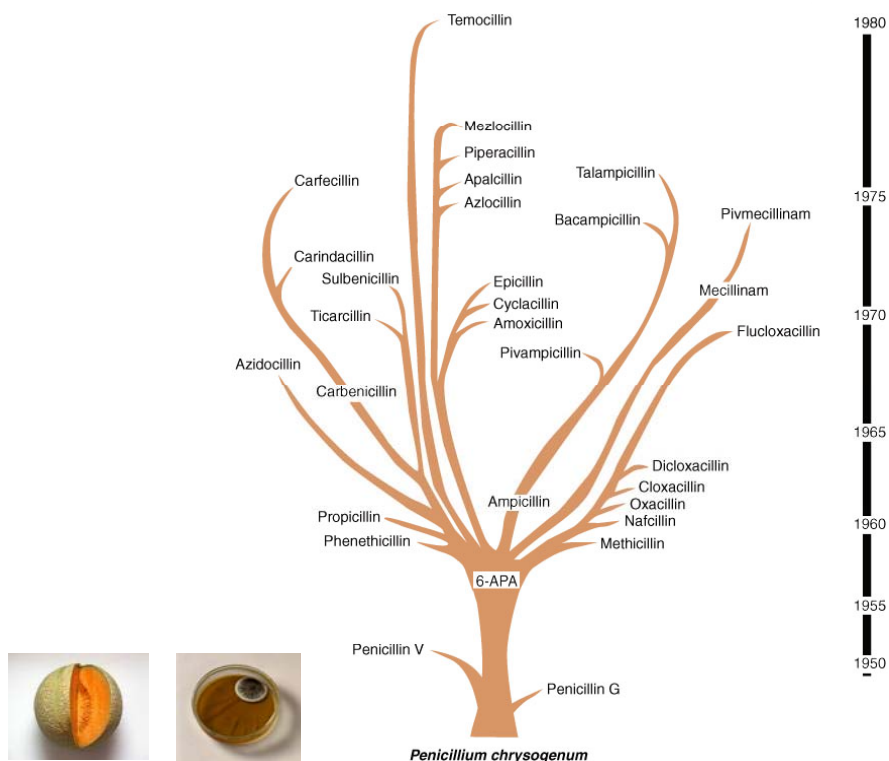
Nobel Prize in Chemistry 1964
for X-ray crystallographic
structure determination of
important biochemical
molecules.



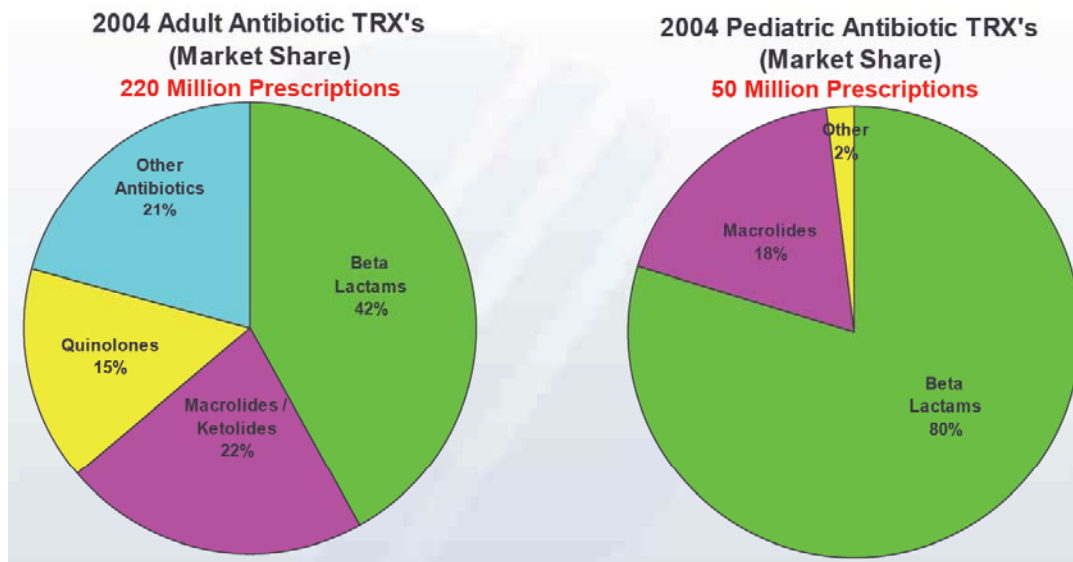
In 1944, Hodgkin crystallized penicillin and determined its structure by X-ray crystallography, solving a controversy around the chemical constitution of the antibiotic and paving the way for discovery of improved antibiotics by modification of the natural products and total synthetic approaches.

Since then, penicillins and other β -lactam drugs have become the single most important class of antibiotics.

First Antibiotic from a Natural Source: Penicillin & β -Lactams



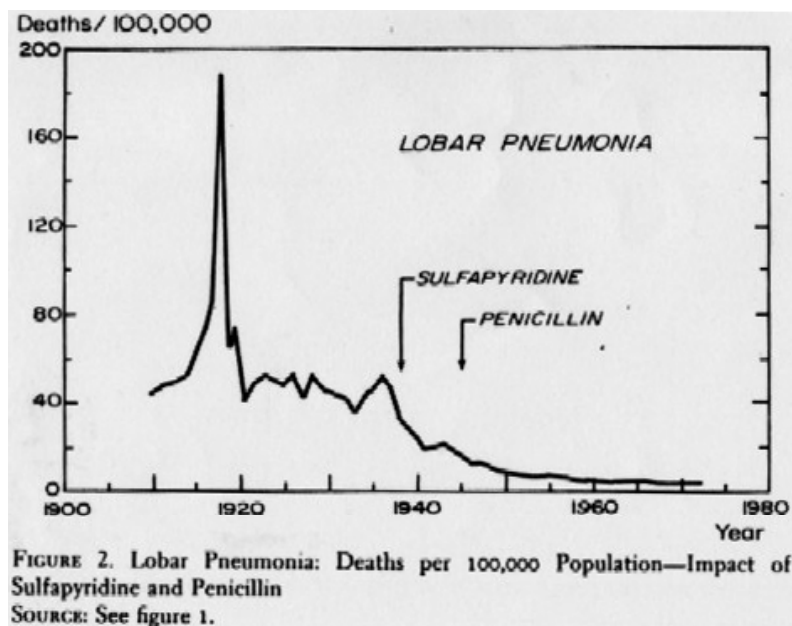
First Antibiotic from a Natural Source: Penicillin & β -Lactams Today



(Source: IMS Health, National Prescription Audit)

(The comparatively larger share of β -lactams in the pediatric prescriptions reflects the good tolerance and safety profile of these antibiotics.)

Impact of Penicillin and Sulfonamides on Mortality



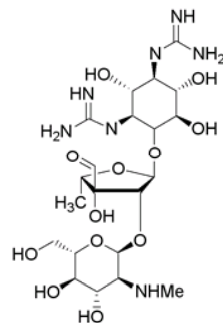
Antibiotics from Systematic Screening: Waksman & Aminoglycosides



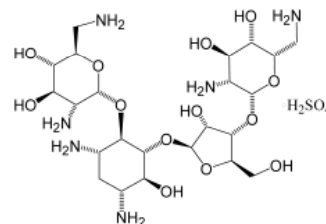
Selman A. Waksman
(New Jersey, 1888-1973)

Worked at Rutgers University where he systematically screened soil bacteria and fungi as source of antibiotics. Discovered a number of new antibiotics, including actinomycin, clavacin, streptothricin, streptomycin, grisein, neomycin, fradycin, candidin, and others.

Nobel Prize in Medicine 1952 for discovery of streptomycin, the first antibiotic effective against tuberculosis.



Streptomycin
(1943)
from *Streptomyces griseus*



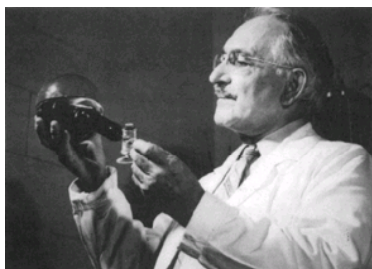
Neomycin B
(1948)
from *S. fradiae*

Aminoglycoside antibiotics bind to the decoding region of bacterial ribosomal RNA and interfere with the accuracy of protein synthesis.

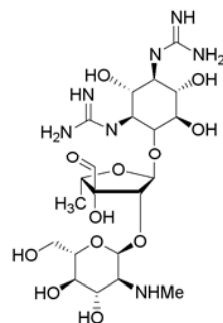
Funded by Merck & Co, Waksman adopted methods of mass screening that had been successful in the German dye industry (also -> Salvarsan) to identify new antibacterial natural products.

Waksman's laboratory performed the microbiological screening, and Merck scientists took over chemistry, pharmacology and large scale production.

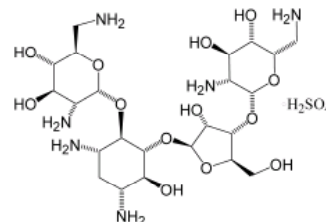
Antibiotics from Systematic Screening: Waksman & Aminoglycosides



Selman A. Waksman
(New Jersey, 1888-1973)



Streptomycin



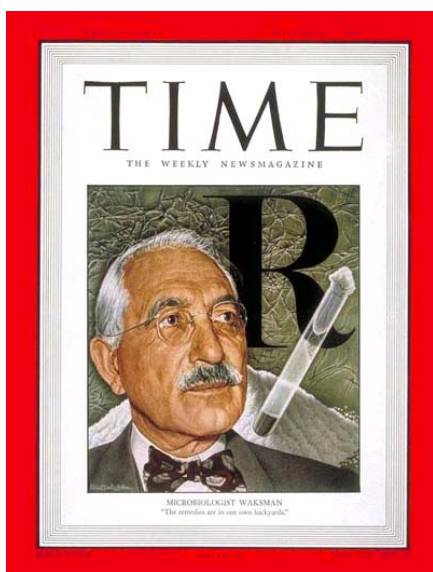
Neomycin B

Thus, in the Summer of 1943, the shelves in the New Brunswick laboratory began to fill up with jars of soil being enriched by the addition of pathogens; and the incubator became crowded with petri dishes in which individual bacteria were being pitted against those same pathogens....

About three months after the start of this program, two strains of *Streptomyces griseus* were isolated that showed great promise. They were found by the agar plate method (there either had not yet been time for the soil enrichment process to show any results or that method failed in this instance), and only after several thousand microbial types had been tested.

Sam Epstein and Beryl Williams, *Miracles from Microbes: The Road to Streptomycin* (New Brunswick, N.J.: Rutgers University Press, 1946), p. 136.

Antibiotics from Systematic Screening: Waksman & Aminoglycosides



Nov. 1949

The Merck company agreed to turn over the rights for streptomycin to Rutgers University, which in turn licensed companies for production. In the late 1940s eight pharmaceutical companies began mass producing streptomycin.

An estimated \$1 million worth of the drug was provided for one the largest clinical studies of a drug ever undertaken, a study involving several thousand tuberculosis patients.



Streptomycin production at Merck in the 1940s

Two years after World War II, the two antibiotics commercially developed by Merck & Co, penicillin and streptomycin, account for half the sales of all drugs.

Timeline of Antibiotics Discovery: Introduction Into the Clinic

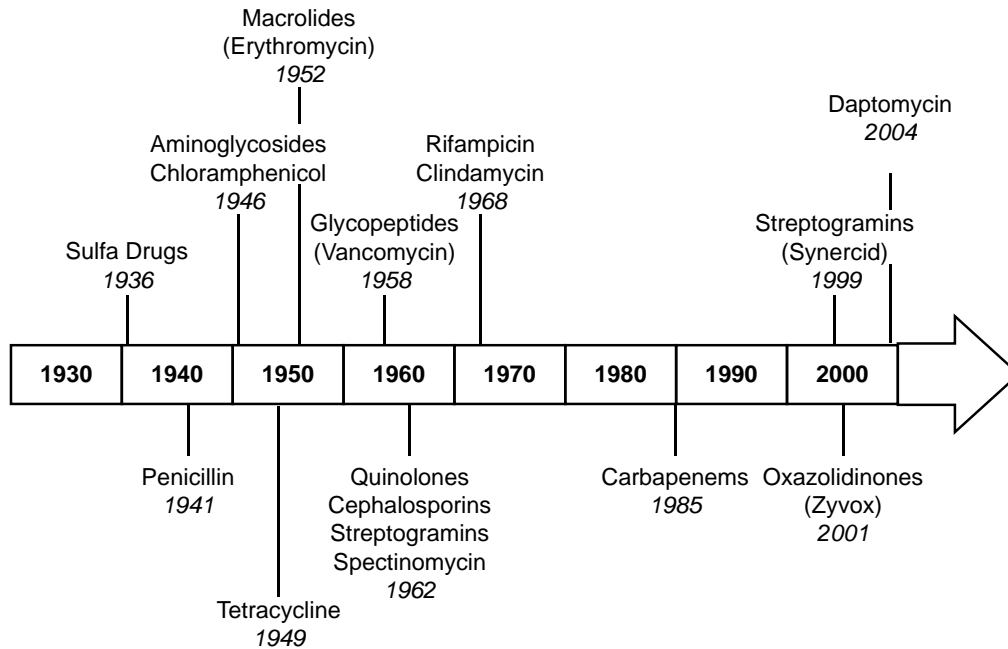
Table 1 – Antibiotic class with approximate year of clinical introduction, lead derivation, example of drug and mechanism of action

| Antibiotic class | Introduction | Derivation | Example | Mechanism |
|-------------------|--------------|------------------------|-----------------------|-------------------------|
| Sulphonamide | 1935 | Synthetic | Sulfapyridine | Antifolate |
| β-Lactam | 1941 | NP-derived | Penicillin | Bacterial cell wall |
| Bacterial peptide | 1942 | NP-derived | Bacitracin | Bacterial cell wall |
| | | | Polymixin | Bacterial cell membrane |
| Aminoglycoside | 1944 | NP-derived | Streptomycin | Protein synthesis |
| Cephalosporin | 1945 | NP-derived | Cephalosporin | Bacterial cell wall |
| Nitrofurantoin | 1947 | Synthetic | Nitrofurantoin | Various |
| Hexamine | 1947 | Synthetic | Methenamine mandelate | Release of formaldehyde |
| Chloramphenicol | 1949 | NP-derived | Chloramphenicol | Protein synthesis |
| Tetracycline | 1950 | NP-derived | Chlortetracycline | Protein synthesis |
| Isoniazid | 1951 | Synthetic ^a | Isoniazid | Fatty acid biosynthesis |
| Viomycin | 1951 | NP-derived | Viomycin | Protein synthesis |
| Macrolide | 1952 | NP-derived | Erythromycin | Protein synthesis |
| Lincomycin | 1952 | NP-derived | Lincomycin | Protein synthesis |
| Streptogramin | 1952 | NP-derived | Virginiamycin | Protein synthesis |
| Cycloserine | 1955 | NP-derived | Cycloserine | Bacterial cell wall |
| Glycopeptide | 1956 | NP-derived | Vancomycin | Bacterial cell wall |
| Novobiocin | 1956 | NP-derived | Novobiocin | DNA synthesis |
| Ansamycin | 1957 | NP-derived | Rifamycin | RNA synthesis |
| Nitroimidazole | 1959 | Synthetic | Tinidazole | DNA synthesis |
| Ethambutol | 1962 | Synthetic | Ethambutol | Bacterial cell wall |
| Quinolone | 1962 | Synthetic | Nalidixic acid | DNA synthesis |
| Fusidic acid | 1963 | NP-derived | Fusidic acid | Protein synthesis |
| Diaminopyrimidine | 1968 | Synthetic | Trimethoprim | Antifolate |
| phosphonate | 1969 | NP-derived | Fosfomycin | Bacterial cell wall |
| Pseudomonic acid | 1985 | NP-derived | Mupirocin | Protein synthesis |
| Oxazolidinone | 2000 | Synthetic | Linezolid | Protein synthesis |
| Lipopeptides | 2003 | NP-derived | Daptomycin | Bacterial cell membrane |

^a Isoniazid is based on the structure of nicotinamide (vitamin B₂).

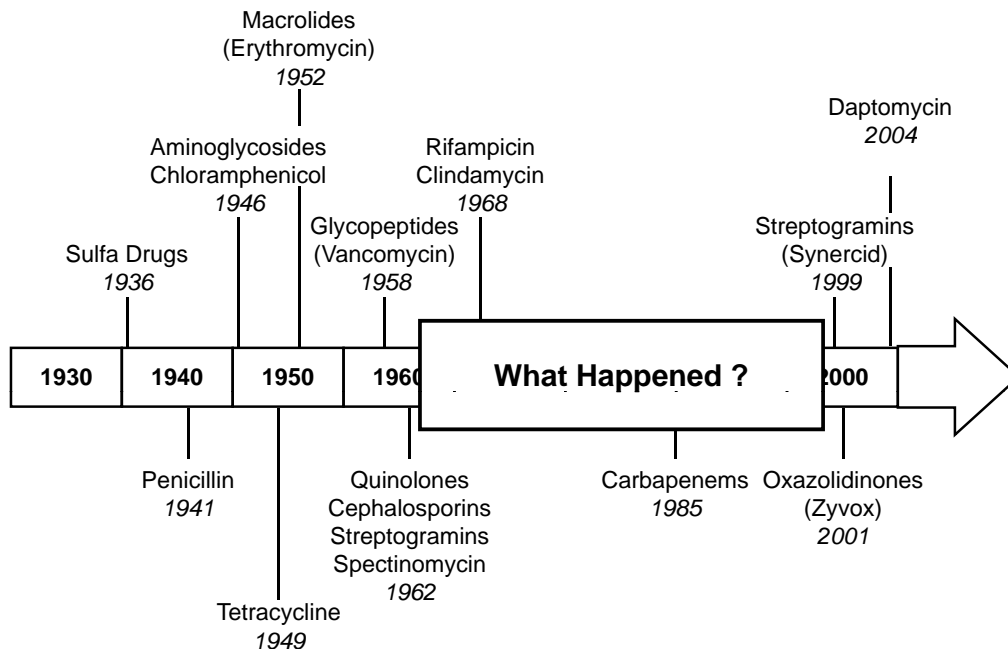
(Source: Biochem. Pharmacol. 2006, 71, 919)

Timeline of Antibiotics Discovery: Introduction Into the Clinic



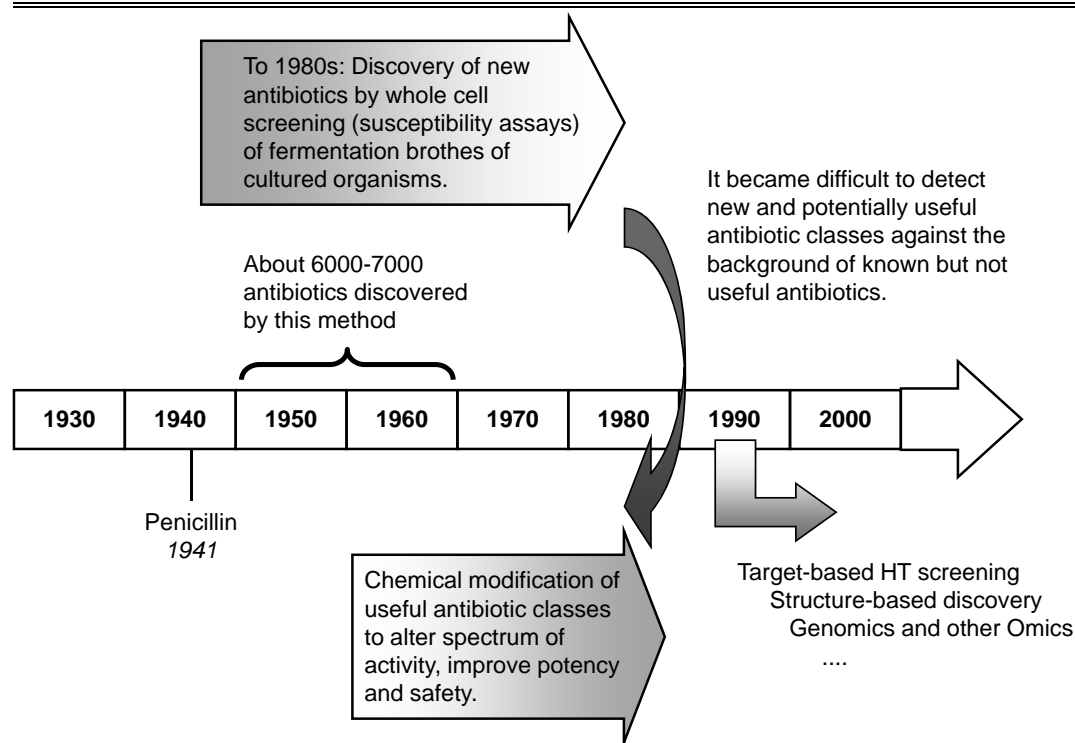
(Antibiotics classes of distinct chemical constitution and mechanism of action)

Timeline of Antibiotics Discovery: The End of the Golden Age (1941-1962)



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Timeline of Antibiotics Discovery: The End of the Golden Age (1941-1962)

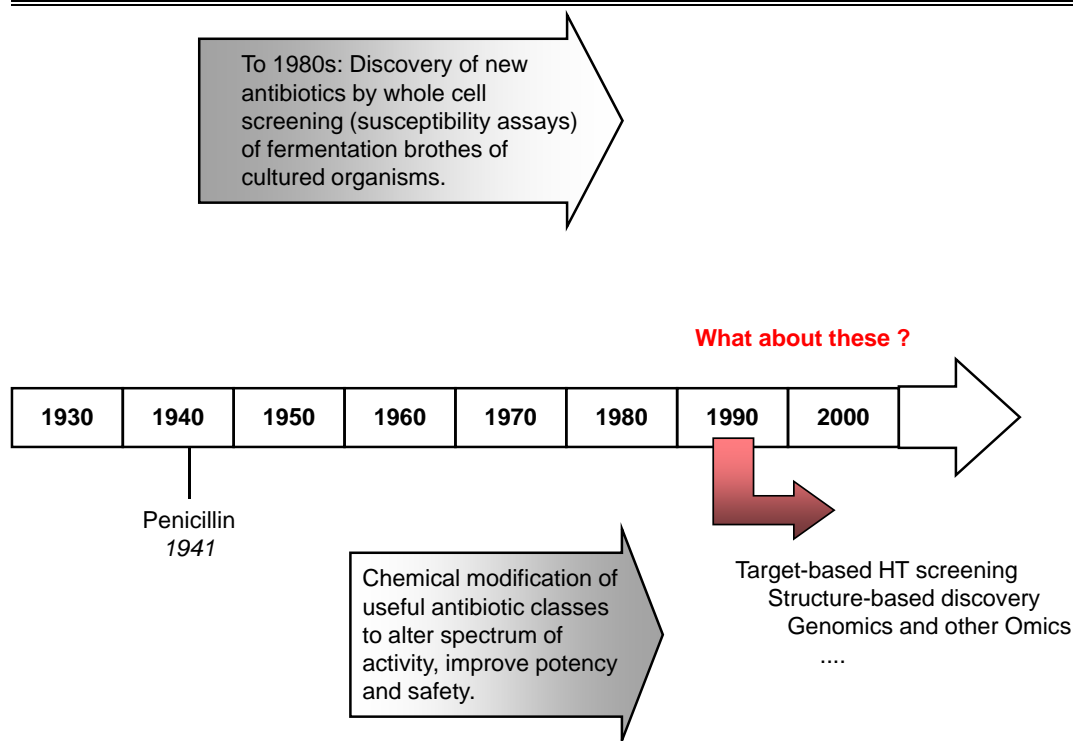
- By the late 1980s a large number of antibiotics were available for medical use at the same time when the discovery of new drug classes by traditional methods was extraordinarily difficult.
- The traditional process of antibiotics dicoverly by whole cell screening of natural sources, purification of individual compounds and structural characterization became an activity of diminishing returns.

and today ...

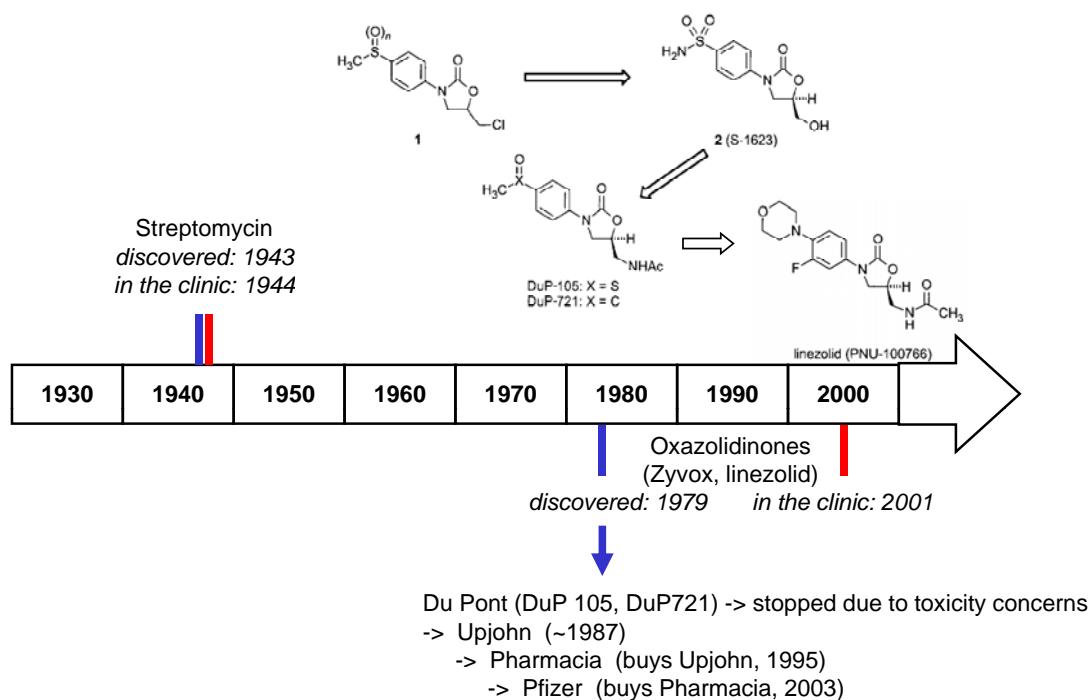
Table 1

| Project therapeutic class | Risk adjusted NPV x\$1,000,000 |
|-----------------------------|--------------------------------|
| Musculoskeletal | 1,150 |
| Neuroscience | 720 |
| Oncology | 300 |
| Vaccines | 160 |
| Injectable Antibiotic (Gm+) | 100 |
| MS- Psoriasis | 60 |
| Liver Transplant | 20 |
| Oral Contraceptive | 10 |

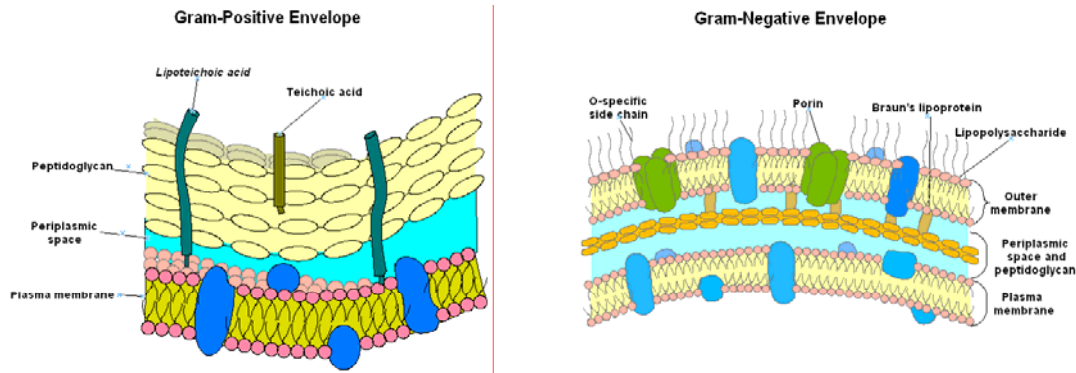
Timeline of Antibiotics Discovery: The End of the Golden Age (1941-1962)



Timeline of Antibiotics Discovery: Tale of Two Cases, Then and Now

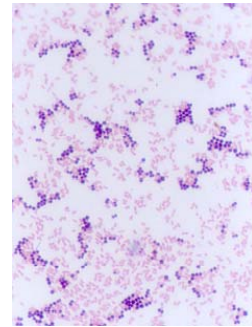


Classification of Bacteria by Gram Staining

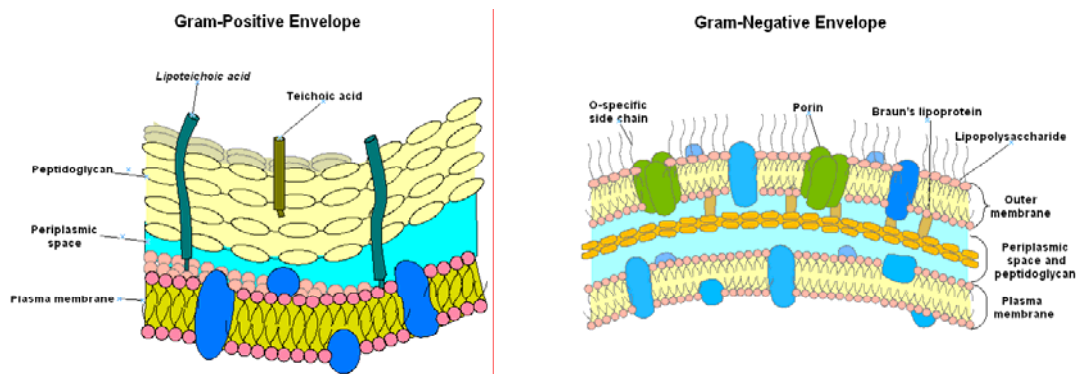


Gram staining is a technique that distinguishes between two groups of bacteria by the identification of differences in the structure of their cell walls (Christian Gram, Copenhagen 1853-1938): gram-positive bacteria remain colored after staining with crystal violet and subsequent washing; gram-negative bacteria do not retain dye.

Cells on a microscope slide are heat-fixed and stained with crystal violet. Then they are treated with an iodine-potassium iodide solution that allows the iodine to enter the cells and form a water-insoluble complex with the crystal violet dye. The cells are treated with alcohol or acetone solvent in which the iodine-crystal violet complex is soluble. Following solvent treatment, only gram-positive cells remain stained, possibly because of their thick cell wall, which is not permeable to solvent. After the staining procedure, cells are treated with a counterstain, i.e., a red acidic dye such as safranin or acid fuchsin, in order to make gram-negative (decolorized) cells visible. Counterstained gram-negative cells appear red, and gram-positive cells remain blue.



Classification of Bacteria by Gram Staining



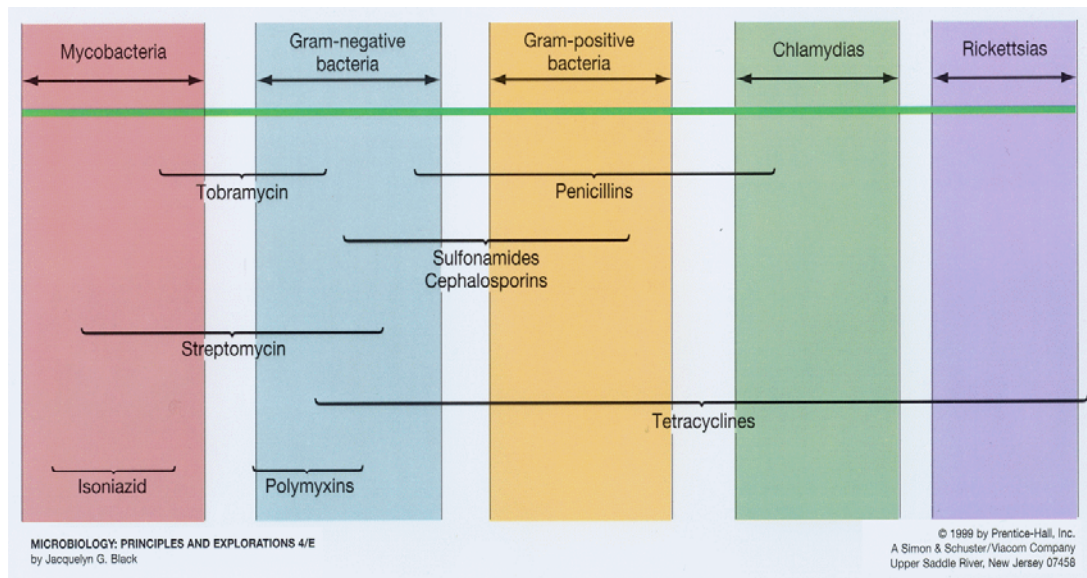
Gram positive:

Staphylococcus aureus
Staphylococcus epidermidis
Bacillus anthracis
Streptococcus spp.
Enterococcus spp.
Clostridium spp.
 ...

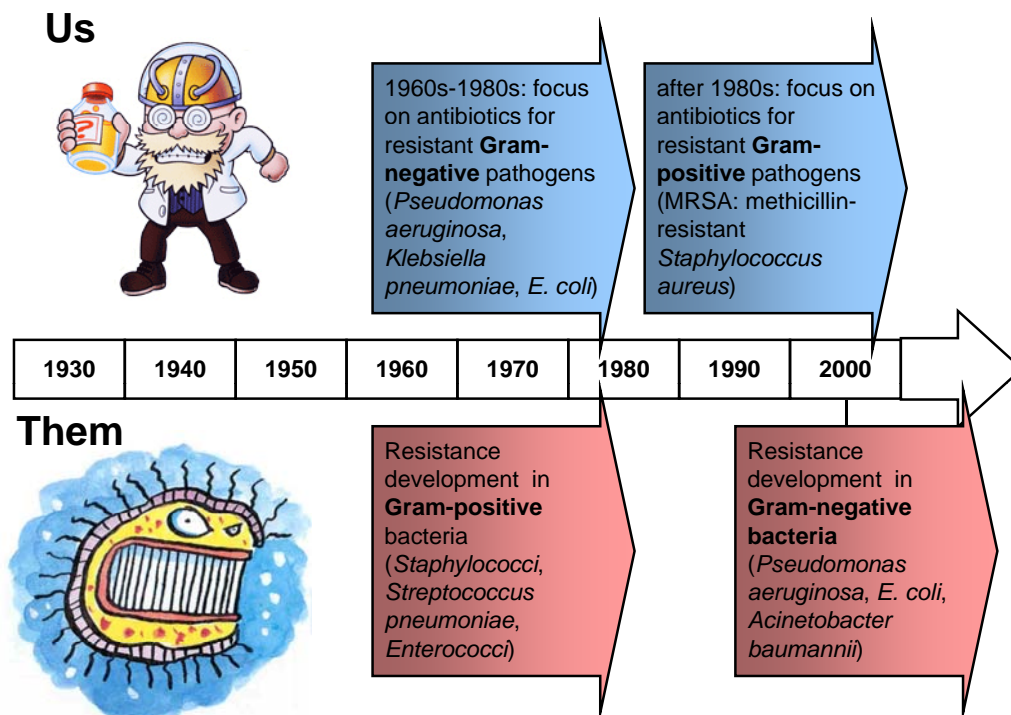
Gram negative:

Escherichia coli
Enterobacter cloacae
Helicobacter pylori
Salmonella enteritidis
Salmonella typhi
Hemophilus influenzae
Pseudomonas aeruginosa
Klebsiella pneumoniae
Legionella pneumophila
 ...

Antibacterial Spectrum of Antibiotics

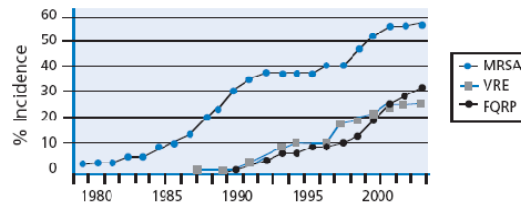


Timeline of Antibiotics Discovery: Chasing After Trends



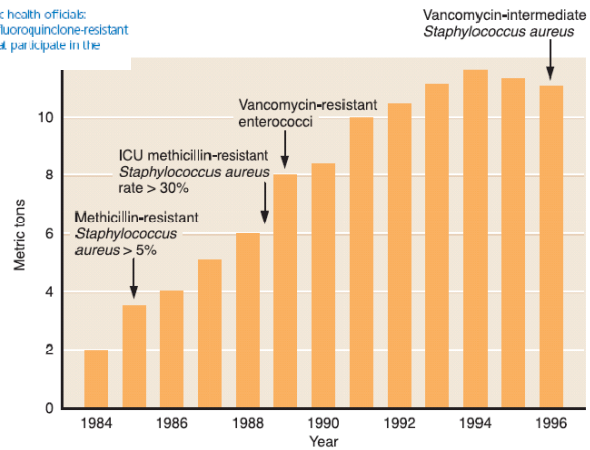
The Race Goes On

Chart 1: Resistant Strains Spread Rapidly



Source: Centers for Disease Control and Prevention

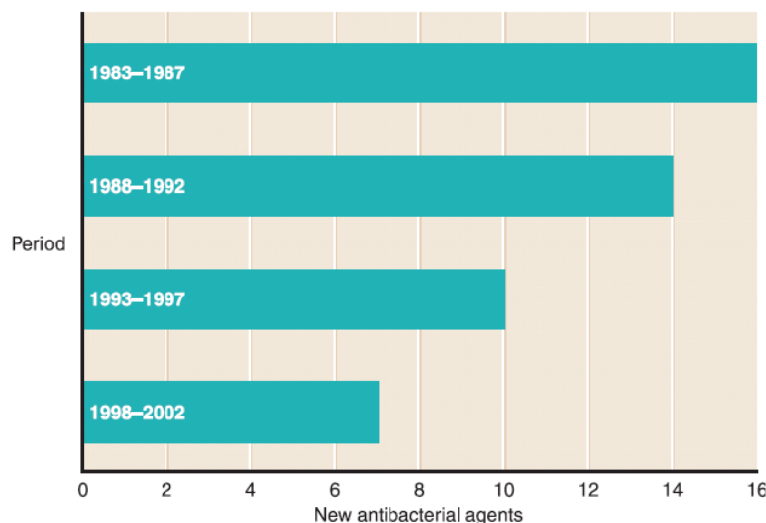
This chart shows the increase in rates of resistance for three bacteria that are of concern to public health officials: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and fluoroquinolone-resistant *Pseudomonas aeruginosa* (FQRP). These data were collected from hospital intensive care units that participate in the National Nosocomial Infection Surveillance System, a component of the CDC.



Resistance creates markets, use creates resistance. Vancomycin use in the United States, 1984–1996.

The Future of Antibiotics?

In 2002, **out of 89** new medicines emerging on the market, **no new antibacterial drugs** were approved. Since 1998, only seven new antibacterials have been approved. Current annual reports for leading pharmaceutical companies (Merck, Pfizer, GlaxoSmithKline, Bristol-Myers Squibb, Aventis, Abbot, AstraZeneca, Lilly, Hoffmann-LaRoche, Johnson & Johnson, and Novartis) list only **4 new antibacterials in the drug pipeline out of 290 agents** listed (or 1.38% of the products in development).



New antibacterial agents approved in the United States per 5-year period from 1983 to 2002.

(Source: ASM News 2004, 70, 275)