Mechanistic Classes of Antibacterials

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An antibiotic is "a chemical substance having the capacity, in dilute solution, to kill or inhibit growth of microorganisms." This definition includes antifungals and antiprotozoals. Antibiotics travel inside the body to fight microorganisms, antiseptics are used externally, and disinfectants are for non-living surfaces.

- Disinfectants
  - Applied to non-living objects
- Antiseptics
  - For external applications to living organisms
- Antibacterials
  - For internal or external use
- Antifungals
  - Kill fungus
- Antiprotozoals
  - Kill protozoans (single-celled eukaryotes)
Antibacterials - A Subclass of Antibiotics

- **Streptococcus pyogenes** - strep throat, rheumatic fever, scarlet fever, necrotizing fasciitis
- **Streptococcus pneumoniae** - (bacterial) pneumonia
- **Escherichia coli** - gastroenteritis, urinary tract infections, sepsis, diarrhea
- **(Methicillin-resistant) Staphylococcus aureus (MRSA)** - Imepetigo, Staph infections, toxic shock syndrome

Top-Selling Antibiotics in the USA

- **Levaquin (Levofloxacin)** by Ortho-McNeil, $1.355 Million
- **Solodyn (Minocycline)** by Medics, $673 Million
- **Doryx (Doxycycline)** by Medics, $673 Million
- **Avelox (Moxifloxacin)** by Merck, $353 Million
- **Ciprodex otic (Cipro and Dexamethasone)** by Alcon, $255 Million
- **Zyvox (Linezolid)** by Pfizer, $223 Million
- **Vanocin (Vancomycin)** by ViroPharma, $192 Million
- **Vancocin** by ViroPharma, $192 Million
Vaccinations and antibiotics can cure or prevent the majority of infectious diseases currently afflicting humanity.

Antibiotic use introduces evolutionary selection pressure to bacteria; Resistant strains are selected for, and cause antibiotics to become ineffective.

Bacterial resistance has been observed for every class of antibiotic introduced, sometimes within one year.

New therapies will be needed.

Table from: Palumbi, S. R. Science 2001, 293, 1786-1790

Table 1. Dates of deployment of representative antibiotics and herbicides, and the evolution of resistance. [Source (75)].

<table>
<thead>
<tr>
<th>Antibiotic or herbicide</th>
<th>Year deployed</th>
<th>Resistance observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>1930s</td>
<td>1940s</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1943</td>
<td>1946</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1946</td>
<td>1959</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1947</td>
<td>1959</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1953</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1955</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1958</td>
</tr>
<tr>
<td>Methicillin</td>
<td>1960</td>
<td>1961</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1961</td>
<td>1973</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1965</td>
<td>late 1960s</td>
</tr>
</tbody>
</table>

- Overkill (multiple antibiotics) has worked well for HIV/AIDS, but not always applicable for bacteria (side effects).
- Direct observation therapy - continue antibiotic dose until no bacteria remain (not practical).
- Use narrow-spectrum antibiotics when applicable.
- Withhold the most powerful drugs - prevented vancomycin resistance for >30 years - difficult business model for pharma.
- Continue to develop new therapies and improve old therapies.

Restricts for this is: Palumbi, S. R. Science 2001, 293, 1786-1790
Bacterial Resistance - Three Flavors

Three types of bacterial resistance to antibiotics have been observed, coming either from random mutation under the selection pressure of antibiotics, or from antibiotic-producing bacteria. Resistance can be spread amongst bacteria via horizontal gene transfer.

- Antibiotic modification (only natural products and semisynthetics)
- Export pumps (efflux)
- Enzymatic target modification

http://www.sciencephoto.com/image/151999/530wm/C0089313-Active_efflux_artwork-SPL.jpg

Paul Ehrlich and Drug Discovery

- Made seminal contributions in histology, haematology, immunology, oncology, microbiology and pharmacology.
- Alongside Ilya Mechnikov, won the Nobel Prize in Physiology or Medicine in 1908 "in recognition of their work on immunity."
- Most famous for his discovery of Salvarsan (arsphenamine, #606), a compound for the treatment of syphilis, which was discovered during the first screen of a library of compounds for pharmaceutical activity, and later part of the first optimization of a lead, becoming the first blockbuster drug and presaging modern drug discovery.

http://pubs.acs.org/cen/coverstory/83/8325/8325salvarsan.html
Antibiotics either target processes that are unique to bacteria - cell wall biosynthesis and folate metabolism - or processes that have different enough machinery to allow selective inhibition of bacterial over human versions - protein biosynthesis and DNA and RNA replication and repair.
Antibacterials Inhibiting Bacterial Folate Biosynthesis

- Prontosil was the first sulfa drug, discovered by Gerhard Domagk while working for Bayer AG.
- Bayer AG hoped to use its expertise in dyes to develop a Ehrlich-style “magic bullet” dye that could be selective for pathogenic bacteria, found Prontosil.
- Immensely successful as the first broad-spectrum antibiotic.
- Bayer AG’s revenue stream was undercut when a team of French scientists found that Prontosil is a prodrug, and becomes sulfanilamide in the body, the patent for which had long ago expired.
- Massive product and marketing of sulfanilamide followed: One preparation, called Elixir Sulfanilamide, was a solution in ethylene glycol. This raspberry-flavored concoction caused over 100 deaths in 1937.
- In 1938, the FDA passed the Federal Food, Drug and Cosmetic Act, requiring safety tests for a variety of product. This is why we do clinical trials for all new medicines today.

Sulfa Drugs - Tetrahydrofolate Biosynthesis in Bacteria

- Prontosil - 1932
- Sulfanilamide - 1936
- Gerhard Domagk Nobel Prize 1939 “for the discovery of the antibacterial effects of prontosil”

http://www.nobelprize.org/nobel_prizes/medicine/laureates/1939/domagk.html
http://www.fda.gov/AboutFDA/WhatWeDo/History/ProductRegulation/SulfanilamideDisaster/default.htm
Sulfa Drugs - Tetrahydrofolate Biosynthesis in Bacteria

7,8-Dihydropteroate Synthase

7,8-Dihydropteroate

Sulfamethoxazole combination marketed as co-trimoxazole

Trimethoprim

7,8-Dihydrofolate

5,6,7,8-Tetrahydrofolate

Success and Failure with Bacterial Metabolite Biosynthesis

Folate is an essential nutrient for humans, they cannot synthesize it.

Bacteria must synthesize folate, and cannot obtain it from their environment.


The Four Major Targets of Antibiotics

Antibacterials that Target Cell-Wall Biosynthesis

Images from: http://fernness.com/science-01.html
Antibacterials that Target Cell-Wall Biosynthesis

The cell wall is called murein, from the Greek word for wall, and the enzymes involved in its construction are hence named MurA, MurB, etc.

β-Lactams: Penicillins, Cephalosporins and Carbapenems

Covalent intermediates have half-lives from several hours to many days


Discovery of Penicillin: Nobel Prize 1945

Sir Alexander Fleming

Sir Howard Florey

Dr. Ernst B. Chain

assisted by Sir. Robert Robinson

http://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/

β-Lactams: Penicillins, Cephalosporins and Carbapenems

Penicillin G (penicillin)

Cefepime (cephalosporin)

Thienamycin (carbapenem)

Structural modifications allow decreases susceptibility to β-lactamases.
β-Lactams: Resistance Mechanisms - β-Lactamases

Costs of over $30 billion per year due to β-lactamase-mediated resistance


β-Lactams: Resistance Mechanisms - β-Lactamase Inhibitors

Clavulanic acid (right) shows minimal activity against transpeptidases (penicillin-binding proteins) but is highly active against β-lactamases.

A combination of Clavulanate and Amoxicillin is marketed by GlaxoSmithKline as Augmentin (and by Pfizer as Clavamox)

Aminoglycosides: Vancomycin and Teicoplanin

vancomycin

“The antibiotic of last resort”
Discovered in soil sample from Borneo, isolated by Eli Lilly chemist Edmund Kornfeld
>30 years before resistance observed (1953 to 1987)

Marketed by Sanofi-Adventis
Approved in 2009

Removing this single hydrogen-bond interaction reduces vancomycin affinity for the terminal dipeptide by 1,000-fold. Replacing the terminal D-Ala with D-Ser reduces affinity by 6-fold.

Boger, 2011 showed that activity can be returned by replacing the amide with an amidine.

The Four Major Targets of Antibiotics

Antibiotics Blocking Bacterial Protein Biosynthesis

Figure from: Steitz, T. A. Nat. Rev. Mol. Cell Biol. 2008, 9, 242-253
Antibiotics Blocking Protein Biosynthesis: Erythromycin

Erythromycin

Erythronolide (Erythromycin aglycone)

Telithromycin

First isolated by Eli Lilly Scientist J. M. McGuire from soil samples collected by A. Aguilar which contained Saccharopolyspora erythraea, a species of actinomycete (major group of antibacterial-producing bacteria)

Acid-instability hampered widespread application of early derivatives; Mono-deglycosylation, C-6 methylation and carbamate introduction aided in newer generations.


Erythromycin Binds to the Ribosome Exit Tunnel

Image from: http://www.weizmann.ac.il/sb/faculty_pages/Yonath/10A-1.jpg

Figure from: Schlünzen, F. et. al. Nature 2001, 413, 814-821
**Antibiotics Blocking Protein Biosynthesis: Erythromycin**

Colored lines indicate groups that are <4.4 Å apart.

- Dimethylation or adenosine replacement causes resistance.
- Humans have guanosine.

**Erythromycin Resistance in Bacteria**

Antibiotics Blocking Protein Biosynthesis: Linezolid (Zyvox)

- Antibacterial effects of oxazolidinone originally discovered by DuPont, abandoned due to toxicity issues. Pharmacia/Upjohn later salvaged the product and (after being incorporated by Pfizer) released Zyvox in 2000.
- To date, Zyvox is the only oxazolidinone clinically approved, but many others are currently in clinical trials.
- Represents the first widely-used novel antibiotic structural class since the 1960s (fluoroquinolones).
- Differences in binding site between other protein biosynthesis inhibitors prevents cross-resistance.


Antibiotics Blocking Protein Biosynthesis: Tetracycline

- Macrolides and oxazolidinones interact with the 30S ribosomal subunit.
- Tetracyclines interact with the 30S ribosomal subunit.

Antibiotics Blocking Protein Biosynthesis: Tetracycline

Chlorotetracycline (aureomycin) was the first tetracycline antibiotic, discovered in a soil sample in 1948 (again biosynthesized by actinomycetes).

Oxytetracycline (terramycin) was subsequently discovered in 1949 by a nascent Pfizer, and was the subject of the first mass-marketing drug campaign. This drug put Pfizer on the map.

R. B. Woodward and Pfizer collaborated to solve the structure of terramycin, mostly succeeding (mis-assigned one stereocenter).

Hydrogenation of aureomycin gave the deschloro product, which maintained activity, and was one of the first semi-synthetic antibiotics.


Novel Tetracyclines from Tetraphase

Andrew G. Myers  
Professor of Chemistry  
Harvard University

Fully synthetic tetracyclines overcome bacterial resistance problems in ways that semisynthetics are unable to.

Business plan includes developing new broad spectrum antibiotics as well as narrow-spectrum inhibitors.

Tetraphase Pipeline

http://tphase.com/
Antibiotics Interfering with DNA Replication and Transcription

Nalidixic acid was the first synthetic quinolone antibiotic, formed as a byproduct during chloroquine (anti-malarial) manufacture in the early 1960s.

Ciprofloxacin, or Cipro, is a second-generation fluoroquinolone, patented in 1983 by Bayer A. G. and receiving FDA approval in 1987. Fluorine substitution at the 6-position (common by this point) provides activity against both gram-negative as well as gram-positive bacteria. Sales were $242 million in 2008.

Levofloxacin (Levaquin or Tavanic) and Moxifloxacin (Avelox or Avelon) are fourth-generation fluoroquinolones, generating $1,355 and $353 million in revenues in 2010, respectively.

Quinolones Stabilize the DNA-Gyrase Covalent Intermediate

Quinolones (and coumarins) cause accumulation of the doubly-cut covalent DNA-gyrase intermediate. The nature of the binding is uncertain (resistance hotspots on gyrA and parC subunits, may bind altered conformation of DNA)

Future Directions of Antibiotics Research - Molecules

Synthetic (top) and natural-product derived (left) antibiotics that have been launched since 2000

PA-824 is a tuberculosis antibiotic, the process-scale synthesis of which was developed at Princeton University

Molecules currently in phase III clinical trials

Butler, M. S.; Cooper, M. A. *The Journal of Antibiotics* 2011, 64, 413-425

Future Directions of Antibiotics Research - Targets

- **Fatty acid biosynthesis** - works well for antiseptics, unlikely to be applicable for antibacterials (environmental uptake).

  ![Fatty acid biosynthesis](image)

- **Two-component signal transduction** - unique bacterial system to modify behavior based on external stimuli.

  ![Two-component signal transduction](image)

- **Efflux blockers** - Target specific, not general.

- **Quorum sensing**

- **Oxidative stress repair**


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Quorum Sensing as a Novel Antibacterial Target

- Two strategies may be possible to use quorum sensing as an antibacterial strategy:

  Develop molecules that turn on QS-regulated genes early, allowing the immune response to eliminate the now-revealed intruder.

  Develop antagonists that prevent QS-regulated genes from being turned on, preventing virulence.

  ![Quorum sensing](image)

Bonnie Bassler
Princeton University

check out TED talk!

Quinolones Stabilize the DNA-Gyrase Covalent Intermediate

Studies non-linear effects in systems biology
In 2007, showed that all bactericidal antibiotics kill bacteria by a common mechanism, oxidative damage from hydroxyl radicals
Suggests targeting RecA in order to potentiate current antibiotic therapies

James J. Collins
Professor of biomedical engineering at Boston University

RecA - involved in DNA repair
Inhibitors currently being developed by Scott Singleton, professor at UNC


Collins, J. J. et. al. Cell. 2007. 130. 797-810

Nitric Oxide as Antibiotic Protection for Bacteria

Evgeny Nudler
Professor of Biochemistry
NYU Langone Medical Center

