How Antibiotics Were Discovered (and Why We Need More)

By Sarah H. Kerns

Antibiotic medications are a familiar component of any medicine cabinet and many doctor visits. The evening news asserts that they are over-used and need to be limited, and multi-drug resistant infections are mentioned ominously. Why? What are antibiotics all about, how does antibiotic resistance work, and why do we care?



Discovery and Development of Antibiotics

The recognition of habitat competition is central to understanding antibiotics. The first modern discovery of antibacterial compounds is credited to Scottish biologist Alexander Fleming in the 1920s who noticed that fungus accidentally growing on plated bacteria inhibited bacterial growth. He noted that microbial competition for resources was the basis of this inhibition: microbes produce compounds which kill or disable other organisms within their local environment to enhance their own access to resources. These early observations form the basis and guiding principle of antibiotic development.

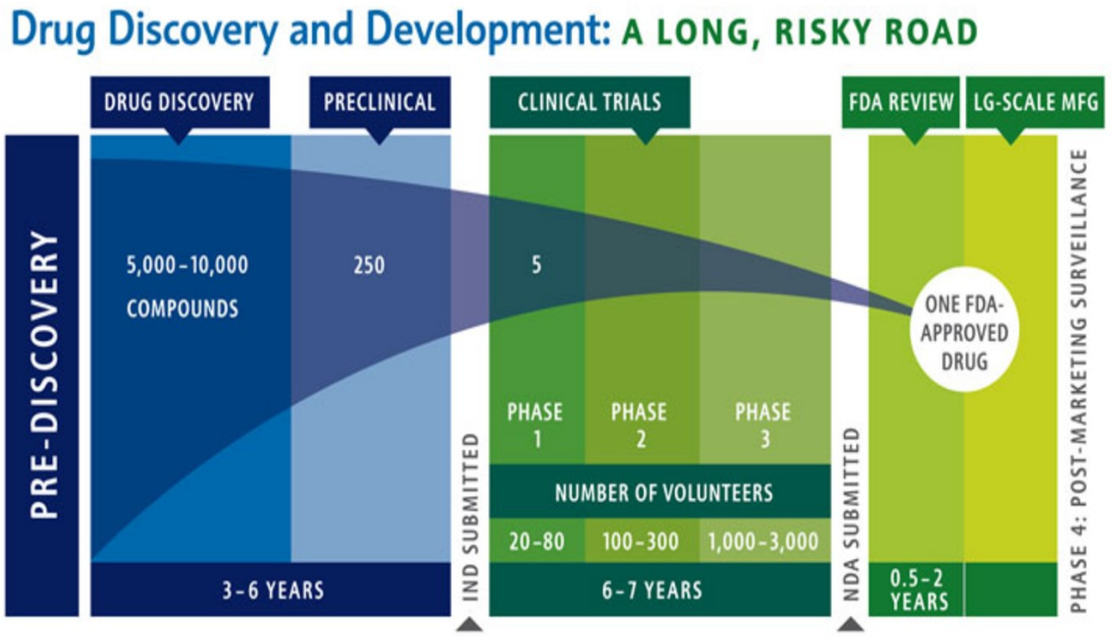
Later scientists discovered that antimicrobial metabolites, while varied in mode of action, are similar in purpose: they disrupt bacterial cell formation. For example: penicillin, made from the antibacterial fungus that Fleming discovered in 1928, is a cell wall inhibitor which kills gram positive bacteria. It does this by interfering with the formation of the bacterial wall during cell division which causes the cells to lose structural integrity and break apart, or lyse. The second antibacterial molecule isolated was the first released as medication. Its sulfa compound inhibits bacterial reproduction by interfering with the synthesis of folate, thereby disrupting cell division. This process, while still attacking reproduction, does not cause lysing which kills the cell, but rather it stalls the reproductive process, disallowing cell division. Because of this, it is categorized as bacteriostatic rather than bactericidal. Other antibiotics were discovered, along with other modes of action, but no matter the mode, the purpose of disrupting bacterial reproduction is consistent.

With these early successes in mind, scientists began looking for newer, more specific antibiotics with systematic search methods, called platforms. One such platform, developed by biologist Selman Waksman, was responsible for major antibiotic discoveries in the 1940-60s, ushered in the Golden Age of antibiotic discovery, and earned Waksman a Nobel prize. He methodically cultivated soil organisms called actinomycetes, tested them for antimicrobial properties, and isolated the metabolites responsible for their action. The first step was growing bacteria from collected soil samples, under resource-rich conditions. He then added them to pathogenic bacteria, to see whether they inhibited pathogenic growth, much the way Pasteur had accidentally grown bacteria-inhibitory penicillin from fungus. Thousands upon

thousands of bacterial plates and samples were grown and tested by Waksman and his team.
Waksman's plates were not specific, instead containing many organisms in each sample. In order to be useful as medication, however, not only the producer strain but also the compound itself had to be isolated. Therefore, once inhibitory colonies were located, they were removed and grown on individual plates. Then, their bacteria-killing chemical metabolites were collected and tested (independent of their producer) to assure repeatability, and these metabolites were chemically sorted based on physical characteristics such as polarity, size, and protein content. Waksman's goal was to isolate the specific small compounds responsible for the bacteria-killing properties.

Once these individual compounds were isolated, they were tested for usefulness in pharmaceutical applications. First, minimum inhibitory concentration (MIC) was determined. This is an essential first step, because a high MIC indicates a compound possibly too weak to kill bacteria *in vivo* and therefore not useful as a pharmaceutical without modification. MIC also helps clinicians to understand possible dosing and administration limitations. Next, safety for use with animal cells was tested using live cell cultures, and finally, animal and human trials were conducted to determine toxicity and side-effects in living organisms.

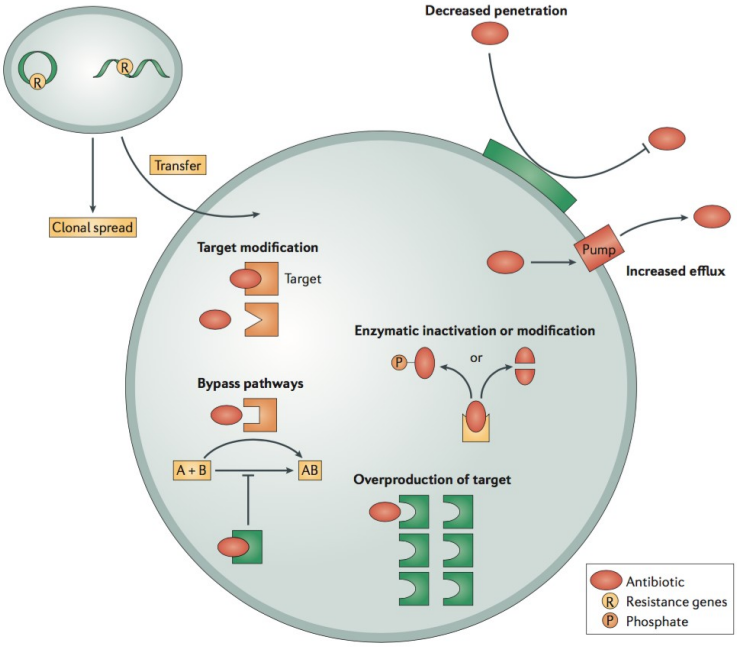
The drug development process is funnel-like, and has decreasing number of useful results surviving each step. Discovery of appropriate compounds happen rarely, but Waksman's team eventually discovered, isolated, and brought to market several important antibiotics of varying usefulness, including Streptomycin, a protein synthesis inhibitor successfully used to treat tuberculosis. (image: Pharmaceutical Research and Manufacturers of America.)



How Does Bacterial Resistance Work?

Other scientists employed different natural discovery platforms, with varying success, and new antibiotic compounds were discovered and developed into medicines, but as the use of antibiotics expanded, physicians and scientists began to see a loss of effectiveness from antimicrobials that had previously worked well. Investigation indicated that microbes were evolving ways to subvert the antimicrobials: they were developing resistance. Scientists were divided on how to study this new issue, with some working to make new and better antibiotics to combat resistant bacteria, and others to understand how the resistance had developed, so it could be addressed both in the lab and in the medical community.

In order to approach both these battlefronts, mutants needed to be grown, and the way they were created in the lab gave early clues as to how resistance might be addressed in the community. This process is the source of the many 'finish all your medications' warnings heard in news and doctors' offices. To create resistant mutants, one must treat bacteria with an effective antibiotic at a slightly sub-MIC level. This amount of antibiotic will kill many, but not all of the bacteria. The surviving bacteria are those of the group that are hardiest, and most resistant to the drug. They are collected and re-grown, using increasing levels of antibiotic, but always leaving the very most hardy bacteria alive to regrow. After many generations, these hardy bacteria are invulnerable to the original antibiotic. They are antibiotic resistant. Once developed in the labs, such mutants were studied to understand their mechanism of survival and learn to disable it.



The ways in which bacteria protect themselves from antibiotics all have the same goal of limiting antibiotic access, but differ wildly. The six primary changes bacteria make to survive chemical attack are:

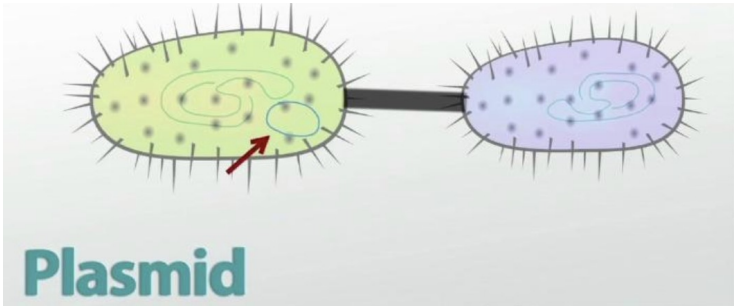
(1) change in the composition of the cell wall to limit antibiotic penetration,

(2) increase in production of efflux pumps in the cell wall to pump antibiotic compounds from the interior of the cell,

(3) alteration of targets on the cell so that the antibiotic no longer connects with/interferes with the natural target,

(4) increase in number of targets, so that normal functions can occur even in the presence of antibiotic compounds,

(5) development of new metabolic pathways to replace those inhibited by the antibiotic, and (6) production of enzymes to disable the antibiotic. (Image: Bacterial methods of drug resistance. Kim Lewis (2015). Platforms for antibiotic discovery. Nature Reviews.)



These structural changes are made through mutations in the cells' genetic information: small accidental changes in base pairs that confer better survival and are thus passed on to the next generation of bacteria. Sometimes useful mutations are transferred from bacteria to bacteria via plasmids (pieces of extra-chromosomal DNA) which code for the helpful trait. Plasmids can be passed on to daughter cells during reproduction, or between adult cells by conjugation pili, which are bridges of cytoplasm stretched between two cells for the purposes of trade and communication These temporary channels represent a fast and effective way to confer immunity once developed.

In response to the growing number of drug-resistant strains, scientists have sought to find new and different antibiotics with the goal of killing specifically, and completely, to eliminate resistance. In the early 1960s, the first entirely man-made antibiotic family was developed: quinolones. The creation of drugs using this synthesis-and-test-based platform was systematic, like Waksman's, but otherwise diametrically opposed to the previous discovery-based methodology of antibiotic development. Starting with a desired action and a central base molecule, or pharmacore, chemical additions were made at one or many points on the surface of the base-molecule to alter its antimicrobial action, and then tested for efficacy against pathogenic and resistant strains. This yielded several drugs used to treat resistant bacterial strains by interfering with DNA replication required for their reproduction, such as ciprofloxacin (cipro). While the drugs were temporarily effective, resistant mutants to fluoroquinolones developed even more quickly than those to naturally- derived antibiotics, and (even worse) resistance to any single fluoroquinolone conferred resistance to all.

Why Do We Care, and What Are We Doing?

Antibiotic resistance is a growing problem, with bacterial innovation far outstripping human discovery. We are just not finding new antibiotics fast enough. Multi drug resistant strains exist in many bacteria, and hospitals and treatment centers which are hotspots of treatment and exposure, are breeding-grounds for continued multi-strain mutation. Although physicians and citizens alike have begun acting to reduce over-use of antibiotics, many worry that it is too late and fear a return to the dark ages of polio, tuberculosis and plague without any way to treat them. It is a nightmare scenario.

Scientists and physicians are working diligently to both stem the formation of resistant strains, through limiting and coordinating how certain drugs are used, and through new avenues of investigation. Several promising studies are in process, for instance: Waksman's effective platform has been revived (using stressed growing conditions rather than abundance) with the hope of uncovering secondary, defensive, metabolites to create new families of antibiotic pharmaceuticals. Studies of human gut bacteria are yielding interesting leads both for strengthening innate defenses[9] and creating new ones, and immunologists work to create vaccines which will allow our own bodies to attack invaders. Many fine minds are spending countless days and hours in a race against these microscopic beasties and their tenacious evolution. The question is: will it be enough?

Resources:

1- https://en.wikipedia.org/wiki/Dihydropteroate_synthase_inhibitor
2- Platforms for antibiotic discovery. Lewis, K (2015). Nature Reviews.
3- https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/selmanwaksman.html
4- Asif, M., A Mini Review on the Study of New Broad-Spectrum Antimicrobial Fluoroquinolone JNJ-Q2
5- Two New Antibiotic Alternatives That Could Save Lives, Brian Krans on October 18, 2013
6- https://en.wikipedia.org/wiki/Pilus#/media/File:Conjugation.svg
7- A Mini Review on the Study of New Broad-Spectrum Antimicrobial Fluoroquinolone JNJ-Q2, Asif, M.

Questions for review:

1. In general, how do antibiotics kill bacteria.

2. Why is the idea of competition for resources important to antibiotic discovery?

3. What are two broad methods that have proven successful for developing antibiotics?

1A. they interfere with or interrupt the reproductive cycle, causing failure of or disruption to new bacterial cell production.

2A. Due to habitat competition, i.e. the need to compete with bacteria for resources, organisms evolved to produce antimicrobial compounds. These compounds are what we identify, purify and mass-produce for pharmaceutical use as antibiotics.

3A. 1. discovering naturally-occurring antimicrobial compounds (ex: penicillin)and 2. designing (or synthesizing) antimicrobial compounds (ex: quinolones)