

Antibiotics and Antibiotic Resistance

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Abstract- In this paper, to better understand the important phenomenon of antibiotic resistance, I shall begin by a review of antibiotics: their discovery, the history of their development, their modes of action, their clinical activities, and the factors determining response to therapy with antibiotics. I shall then investigate the mechanisms of antibiotic resistance, review some major epidemics due to antibiotic resistance and infectious diseases and antibiotic resistance. Antibiotics do not work all the time, their inappropriate use, nosocomial infections, and possible reservoirs of antibiotic resistant animal organisms causing human diseases will then lead us to an update on the present situation, highlighting the nanopore revolution in genomic sequencing of drug-resistant bacteria, precious nanometals, and look forward to the future of antibiotics.

Index terms- Mechanism of Resistance, Modes of Action, Factors, Protein Synthesis

INTRODUCTION

In 1941, Albert Alexander, a policeman in Oxford, England, was the first patient to ever be treated with an antibiotic. While tending to his garden, he had been scratched by a rose - a scratch that quickly became infected by bacteria, probably *Staphylococcus aureus* with an admixture of various *Streptococci*, and turned septic. The sepsis spread, caused the patient's head to swell with abscesses that necessitated the removal of one eye; in short, he was on the verge of dying. He was treated at the Radcliffe Infirmary by Drs. Howard Florey and Ernst Chain who were brewing up extracts of a mold called

Penicillium chrysogenum and had synthesized a very small amount of penicillin. While having been discovered in 1938 by Alexander Fleming, that drug had never actually been used to treat a human. It was effective in improving the policeman's condition. Unfortunately, not enough penicillin had been synthesized and when the treatment stopped, the sepsis roared back and the patient eventually died. Whereas that story did not end well, millions of other people have since lived because of it, and global health had been transformed.

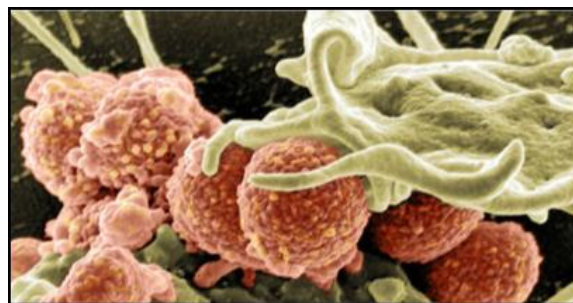


Figure 1: MRSA Being Attacked by a White Blood Cell.

ANTIBIOTICS FROM SCRATCH

For most of the field's history, natural products have been the starting point for new antibiotics. Most of them have been made by chemically modifying natural products in a process known as "semi-synthesis". Every existing antibiotic in a class called "macrolides"— including the commonly prescribed drug Azithromycin—has been made by modifying erythromycin, which was first discovered in a soil sample in 1949. But, as will be abundantly discussed,

some bacteria are developing resistance to these drugs at a startling rate, and semi-synthesis is limited by the difficulty of modifying such complex molecules.

Now, scientists at Harvard University have devised an approach for synthesizing new macrolide antibiotics from simple chemical building blocks [4]. They have synthesized more than 300 new antibiotic candidates, several of which being effective against some of the most stubbornly drug-resistant bacterial strains. This is the first time there is a relatively easy path to synthesize macrolide erythromycin-type antibiotics from scratch (Figure 2).

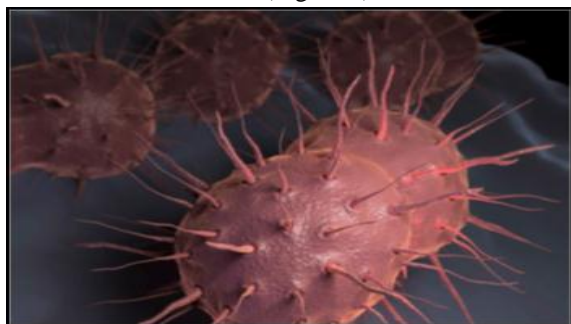


Figure 2: One Type of “Super Bug”.

The macrolides have been synthesized from eight simple chemical building blocks to produce a diverse collection of structures that would be practically impossible to make using semi-synthetic methods. This approach allows for, theoretically, tens of thousands of compounds or more.

Macrolides consist of a macrolactone ring with one or two sugars. “Decorating” these rings with different chemical groups by combining the building blocks in various combinations, Myers’s team created more than 300 synthetic macrolides, including the U.S. Food and Drug Administration (FDA)-approved drug telithromycin and the candidate solithromycin, which is currently being tested in Phase 3 clinical trials.

Next, the researchers evaluated the newly synthesized compounds with a panel of different bacteria, including two strains of MRSA and vancomycin-resistant enterococcus (VRE) isolated from clinical samples. Most of the compounds were effective against garden-variety pneumonia bacteria, and a few of them were more potent than approved antibiotics against MRSA, VRE, and other highly resistant strains. One of the synthetic compounds, called FSM-100573, was especially effective against gram-

negative bacteria, which are traditionally less susceptible to macrolides.

These compounds have yet to be tested pre-clinically. And, as with all antibiotics, bacteria will ultimately develop resistance to them. However, this approach allows scientists to develop an exponentially larger number of new drug candidates. This work represents a paradigm shift in the discovery of novel antibiotics from the macrolide class. Previously, Myers and his colleagues have synthesized novel tetracycline antibiotics, one of which is now a Phase 3 clinical trial candidate.

MODES OF ACTION OF ANTIBIOTICS

Antibiotics can be distinguished on the basis of their mode(s) of action that is on their ability to interfere with the metabolic machinery of microbes. The microbe needs this machinery to thrive, hold itself together, or make duplicate versions of it. Antibiotics are supposed to thwart bacterial – not human – cells through one or more of the following modes of action:

INTERFERENCE WITH MICROBIAL ABILITY TO MAKE CELL WALLS (MODE-1)

This is a common requirement for these organisms to cause infections for without the rigid support of the cell wall; most bacteria simply break open and die, or collapse into, an effective heap. Note, however, that a few varieties of bacteria can actually thrive by constructing outer membranes instead of cell walls, but they rarely can reproduce to create further harm. The key molecular target of these antibiotics is a substance (mucopeptide) that coats the bacterial membrane and gives it its rigidity. Examples include such narrow-spectrum antibiotics as:

- a. Penicillin and its derivatives;
- b. Cephalosporins: Similar to penicillin but with a different part of the process.

Penicillins and cephalosporins can inactivate key bacterial enzymes (peptidases) that synthesize the rigid cellular walls and this all the more effective the thicker the walls. They are remarkably non-toxic at normally used therapeutic dosages because they do not attack human cell walls or membranes which are made from different materials than bacteria.

INHIBITION OF PROTEIN SYNTHESIS (MODE-2)

These are generally more toxic to the human body, and include the broader-spectrum antibiotics such as:

1. Chloramphenicol, an extraordinarily potent antibiotic but also an extremely toxic one for cells of the bone marrow, also causing blood disorders (granulocytosis, pancytopenia) and the so-called “grey syndrome” (weakness, listlessness, gray pallor, hypotension in treated newborn, especially premature infants). Because of this toxicity, chloramphenicol is reserved for serious diseases (typhoid fever, bacterial meningitis);
2. Tetracycline (the broadest spectrum in this category). While less toxic than chloramphenicol, tetracycline avidly binds to calcium, magnesium and other essential minerals in the body;
3. Polymixin (B and E forms): Relatively non-toxic, and available as a topical non-prescription-drug;
4. Erythromycin.

INTERFERENCE WITH PROTEIN OR MEMBRANE SYNTHESIS (MODE-3)

May involve both aerobic and anaerobic bacteria. These antibiotics include the cephalosporins.

INTERFERENCE WITH THE GENETIC SYNTHESIS OR OPERATION (DNA OR RNA) (MODE-4)

These include:

I. Aminoglycosides.

INTERFERENCE WITH THE METABOLIC REACTION(S) (MODE-5)

These include:

1. Trimethoprim;
2. Sulfonamides.

COMBINATION MODES (MODE-6)

Two (or more) chemicals interfere with the same reaction but at different places. Examples include:

1. Trimethoprim;
2. Sulfamethoxazole.

All but the most innocuous localized infections initially pit microorganisms against the host’s natural defenses. Antibiotics usually work by keeping invading microorganisms at bay long enough to allow the host to remove and destroy them.

Antibiotic Clinical Activities

Antibiotic activities are clinically separated between:

1. Suicidal (or -cidal) effect): Kill bacteria outright; and
2. Static effect: Simply stop the growth of antibiotics.

However, in practice, an antibacterial drug can be bacteriocidal at a high dose and bacteriostatic at a lower one. Its effectiveness can be determined through two tests:

- a. Antimicrobial susceptibility test: in which a variety of antibiotic-containing disks are put into tubes or plates containing a growth medium for the bacteria; and
- b. Bacteriocidal (or lethality) test: The quantitative precision of these tests can be misleading as predictors of clinical efficacy for different antibiotics. Further, a bacterium can fail to break open or lyse after exposure to antibiotics (so-called “tolerance” phenomenon), which further complicates the interpretation of these tests. Antibiotics directed against a true pathogen or disease-causing bacteria do not discriminate between it and a beneficial bacterium [5].

FACTORS DETERMINING RESPONSE TO THERAPY WITH ANTIBIOTICS

There are five factors that are considered in evaluating the response to antibiotic therapy:

- a. Initial susceptibility of the bacterium;
- b. Interaction with the host (that is the effective amount of the drug at the site of the infection);
- c. Location of the infection and its severity;
- d. Condition of the patient; and
- e. Use of support measures (e.g., surgery to drain abscesses) Table 3.

Below summarizes the beneficial/detrimental effects of certain antibiotics:

Thus, the pervasive use of antibiotics may cause subtle and often imperceptible changes in the microflora with which we have evolved. This is accompanied with the real possibility of indirect havoc to less physically resilient hosts in one’s closer social environment, including the spread of antibiotics tolerance and resistance.

MECHANISMS OF ANTIBIOTIC RESISTANCE

In 2014, the WHO [6] published its first Global Report on Surveillance titled “Antimicrobial Resistance”. In this report, it focused on antibiotic resistance, that is when bacteria change and antibiotics fail, alerting the world that this phenomenon is no longer a prediction for the future but is happening right now across the world. It subsequently published two Fact Sheets titled “Antibiotic Resistance” and “Antimicrobial Resistance”.

EVOLUTION OF THE MECHANISM OF RESISTANCE

Antibiotics work by inhibiting or killing susceptible microorganisms. Among the billions of germs that make up the population of any given infection, a few somehow withstand a low-level antibiotic assault, survive, and if the level of antibiotic remains low or drops, go on to replicate and form a new “antibiotic-resistant” infection. In other words, bacteria could exist with the innate information needed to resist an antibiotics’ effect even before antibiotics are used.

Resistance could precede the application of antibiotics! It has indeed been demonstrated that chromosomal genes for resistance exist. Antibiotics do not induce resistance (the so-called “induction hypothesis”), as once thought, but rather their inappropriate or/ and inadequate use lead to an evolutionary mechanism wherein microorganisms develop resistance. The antibiotic serves merely to create the conditions that favor the outgrowth of preexisting antibiotic-resistant organisms, and does not induce others to acquire the resistance state. This evolutionary potential of microorganisms (a genetic mechanism) is a key to eradicating the infection if it is incorporated in the therapeutic strategy employed. Evolutionary mechanisms explain both the origins of antibiotic resistance, and the emergence of antibiotic-resistant bacteria.

RESISTANCE SPREADING BEYOND THE INITIAL ORGANISMS

Further, bacteria spread their resistance information beyond the initial organisms. Populations in far flung regions of the world, who have never known or been treated with antibiotics, or been in contact with people who had been treated with antibiotics, were

found to have antibiotics resistance. This demonstrates that resistance is a natural part of the genetic makeup of microbial communities. Information on resistance can be carried on the bacterium’s own chromosome or, when the chromosome breaks off, on these satellites (known as plasmids) leading to two kinds of antibiotic resistance: (a) chromosomal and (b) R-factor mediated. These two mechanisms may be augmented when DNA sequences jump from one DNA molecule to another (called transposons). Transposable resistance may be the most rapid and dangerous form of dissemination of all, since it permits resistance genes to move readily between chromosomes and R-factors and, hence, into the microbial world at large. Further, R-factors can also duplicate themselves within the bacterium itself, making multiple copies of their own chemical instructions thereby augmenting the hazard of antibiotics use.

Further complications are (a) the link between the genes for resistance and those for making some bacteria more virulent, (b) the close physical association between different resistance genes, and (c) the replication of R-factors independently of the bacterial chromosome while inside the bacteria. In other words, the forces that enhance the spread of resistance to one antibiotic simultaneously incorporate resistance to others.

MULTI-ANTIBIOTIC RESISTANT BACTERIA OR SUPERBUGS

Bacteria resistant to one or more antibiotics have been termed “superbugs” (Figure 3). The WHO defines and describes a superbug thus:

“Antimicrobial resistance occurs when microorganisms such as bacteria, viruses, fungi and parasites change in ways that render the medications used to cure the infections they cause ineffective. When the microorganisms become resistant to most antimicrobials they are often referred to as “superbugs”. This is a major concern because a resistant infection may kill, can spread to others, and imposes huge costs to individuals and society”.

Superbugs make it difficult to treat or cure infections that once were easily treated. The antibiotics have lost their ability to control or kill bacterial growth. The bacteria can grow even in a sea of antibiotics because the antibiotics do not touch them as they

have acquired the ability to destroy the antibiotics in order to protect themselves. The bacteria have developed genes for resistance and these genes protect them. Genetic mutations might enable bacteria to produce enzymes that inactivate antibiotics or eliminate the target that the antibiotics are supposed to attack. Further, bacteria may have developed resistance to five or six antibiotics so it is not known which antibiotic to use for treatment. The bacteria can accumulate resistance by developing new genes.

An example of such deadly superbug outbreaks took place beginning in 2009 and lasting to the present date in several hospitals (in Florida, Chicago, Seattle, Los Angeles, Pittsburgh, Hartford, and elsewhere). The cause was contamination from specialized medical scopes, particularly duodenoscopes due to drug-resistant infections. Duodenoscopes are especially difficult to disinfect because of trapped bacteria from a mechanism at the tip of the duodenum. Other causes stem from raw sewage discharged from hospitals, directed to distant treatment plants and released as clear water into water surfaces (streams, oceans,). Unfortunately this treated water is not tested after it flows out of the treatment plant for the presence of the superbug carbapenem-resistant enterobacteriaceae (CRE). This was reported (Los Angeles Times, 7 March 2016) for Southern California hospitals, but was also the case for other U.S. Hospitals. As it turns out, the raw sewage is a highly conducive environment that harbors CRE and allows it to proliferate and grow. As the sewage mixes, the antibiotics kill off weaker bacteria, leaving the more lethal ones to thrive. The bugs reproduce rapidly, and different species can swap genes, transferring their ability to withstand the drugs.

CONCLUSION

On our individual skin surface and lining our alimentary tract, we are inhabited by trillions of microorganisms some of which are beneficial and protective. But, where do they all come from? The process begins at birth and continues throughout the individual's lifetime. Their survival can be arrested or their growth inhibited with antibiotics (natural or synthetic). The history of the development of antibiotics was summarized from their discovery in the late 19th century to this day where it continues as

a unilateral quest for chemical rather than homeostatic or immunological solutions. In 2016, a novel approach was devised for synthesizing new macrolide antibiotics from simple chemical building blocks. More than 300 new antibiotic candidates have been synthesized, several of which being effective against some of the most stubbornly drug-resistant bacterial strains. This is the first time there is a relatively easy path to synthesize macrolide erythromycin-type antibiotics from scratch.

Antibiotics can be distinguished on the basis of their ability to interfere with the metabolic machinery of microbes. They are supposed to thwart bacterial – not human – cells through one or more of the following modes of action:

- a. Interference with microbial ability to make cell walls;
- b. inhibition of protein synthesis;
- c. interference with protein or membrane synthesis;
- d. interference with the genetic synthesis or operation (DNA or RNA);
- e. interference with the metabolic reaction(s); or even a combination of some or perhaps all of the above modes.

Antibiotic activities are clinically separated between those that kill bacteria outright (suicidal or -cidal effect) and those that simply stop the growth of antibiotics (static effect). In practice, however, an antibacterial drug can be bacteriocidal at a high dose and bacteriostatic at a lower one. Its effectiveness can be determined through well-known tests which can be misleading as predictors of clinical efficacy for different antibiotics.

Several factors determine the response to therapy with antibiotics:

- a. Initial susceptibility of the bacterium;
- b. interaction with the host;
- c. location of the infection and its severity;
- d. condition of the patient;
- e. Use of clinical support measures.

Antibiotics have also become the keystone to mass production of livestock. Restraint of the unbridled use of antibiotics supplements in animal feed has not abated in the U.S. By contrast, other nations (Great Britain, former Czechoslovakia, Japan) have recognized that animals can serve as reservoirs of potentially dangerous drug-resistant bacteria, and that

contamination of one segment of the ecosystem virtually ensures contamination of others.

Antibiotic resistance is no longer a prediction for the future; it is happening right now across the world. It is putting at risk the ability to treat common infections in the community and hospitals. Without urgent, coordinated action, the world is heading towards a post-antibiotic era, in which common infections and minor injuries, which have been treatable for decades, can once again kill. The inappropriate use of antimicrobial drugs, including in animal husbandry, favors the emergence and selection of resistant strains, and poor infection prevention and control practices contribute to further its emergence and spread.



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REFERENCES

- [1] World Health Organization (2014) First Global Report on Antibiotic Resistance.
- [2] Fymat AL (2016) “The Odyssey of Human Vaccination and Africa”, Presented at the Third International Scientific Conference of the Society for the Advancement of Science in Africa, Nairobi, Kenya (August).
- [3] Fymat AL (2017) “The Odyssey of Human Vaccination and Africa” in Fymat AL and Kapalanga (eds): Science, Technology and Innovation in Africa, Chapter 7, Cambridge Scholars Publishing, England. <http://www.cambridgescholars.com/science-research-and-education-in-africa>.
- [4] Lappe M (1982) Germs that Won't Die, Anchor Press/Doubleday pp: 246.
- [5] McGann P (2016) Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF Plasmid: First Report of mcr-1 in the United States. Antimicrobial Agents and Chemotherapy 60: 8.
- [6] Sommer M, van Schaik W, Imamovic L, van der Helm E (2015) “Rapid Resistome Mapping Using Nanopores Sequencing”, Nucleic Acid Research 45(8): e61.

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