Why Antibiotics Are Not A Cure-All

A General Overview of Antimicrobial Resistance
Summary / Introduction

Antibiotics, members of a drug family known as antimicrobial agents, have been used to treat patients with infectious bacterial diseases for over 70 years. It is impossible to calculate the number of patients who have benefited from their use; however, because these agents have been used for such a long period of time, and unfortunately, often used inappropriately, antibiotic resistant isolates of the original organisms have emerged. In this paper, we will discuss how antibiotics should work and some of the most common mechanisms whereby antibiotic resistance occurs.
Antimicrobial Resistance

Antimicrobial resistance is the condition that occurs when a microorganism evolves and becomes impervious to the exposure of one or more antibiotic drugs. The infectious bacteria that the drugs are designed to kill adapt over time – primarily through genetic mutation – making the antibiotics less effective.

People who become infected with antimicrobial-resistant bacteria are more likely to experience more lengthy hospital stays, more extensive infections, and therefore are more likely to face lethal consequences as a result of their illness.

Classic Methods of Transfer

In order to become resistant to an antibiotic, an organism must first acquire a gene that mediates the resistance. There are many means by which these incredibly adaptable bacteria can obtain a new gene, including horizontal transfer by the

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What Are Antibiotics?
The word “antibiotic” was first used in 1942 by Dr. Selman Waksman to describe substances that are produced by microorganisms and inhibit or kill other microorganisms. This definition has come to exclude any compound that kills bacteria, but is not produced by microorganisms (such as iodine or hydrogen peroxide). It also excludes synthetic antibacterial compounds such as sulfonamides.

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following methods: (1) Conjugation, (2) Point Mutation, (3) Transduction, (4) Recombination, and (5) Transformation.

**Conjugation**

The first method we will discuss is bacterial conjugation. This is a process whereby genetic information (in the form of a plasmid) is passed from one bacterial cell to another, either by F-pili (“F” meaning fertility) (see Figure 1) or by direct cell-to-cell contact (see Figure 2) attaching to the cell.

Conjugation tends to occur between members of the same or related species, but can occur between different genera. Most eubacteria (true bacteria) can conjugate. However, conjugation most commonly occurs with *E. coli* and other members of the Enterobacteriaceae family, as well as *Bacteroides*, enterococci, streptococci, strepto-mycetes, and clostridia. It should not be surprising that this mechanism is often involved in resistance transfer among members of these groups.

Conjugation is often beneficial to the recipient cell because characteristics such as antibiotic resistance, or the ability to use various new metabolites or other abilities, can be passed on from the donor cell. However, the transfer can sometimes prove to be negative, like when bacterial parasites are spread. In the case of antibiotic resistance by this mechanism, the plasmids transferred are called F-plasmids or F-factors.

The F-factor is an episome, which is a plasmid that can integrate into the host bacterial chromosome. In other situations, the plasmid can be a free segment of DNA, a replicon, which can replicate autonomously from the host chromosome.
Recombination
Recombination occurs when extra chromosomal (foreign) DNA is incorporated into the host’s DNA (see Figure 3 on the right).

Point Mutation
A point mutation is a type of mutation that causes the replacement of a single base nucleotide with another nucleotide in DNA or RNA. Often, this term refers to insertions or deletions of a single base pair, which we will discuss in the section about antibiotic resistance.

Transformation
Transformation was the first mechanism of genetic transfer to be discovered in bacteria. It is a process by which bacteria take up fragments of naked DNA and incorporate them into their genomes (see Figure 4 below). Transformation is a mechanism of resistance transfer found in certain bacterial organisms that can take up and stably maintain exogenous DNA at a high rate. These include *Haemophilus influenza*, *Streptococcus pneumoniae*, *Bacillus* species, and *Neisseria* species.

![Figure 3: Incorporation of extrachromosomal DNA into the chromosome occurs by recombination.](image)

![Figure 4: In transformation, a donor cell contributes DNA fragments during cell lysis, which is when they enter and integrate into a recipient / host cell.](image)
Transduction

Transduction can occur in all bacteria and is the transfer of genetic information mediated by bacterial viruses, or bacteriophages. These bacteriophages pick up fragments of DNA from host cells, or elsewhere, and package them into the new bacteriophage particles. The added DNA is delivered to the next infected cell and becomes incorporated into the bacterial genome. A segment of DNA that can produce antibiotic resistance could be incorporated into the phage particle and transferred to subsequent hosts (see Figure 5). Transduction is a process that is often used by molecular biologists to introduce foreign genes into a host cell’s genome.

![Figure 5: Transducing Phage containing donor genomic DNA moves into cell lysis. The Phage is released and infects a recipient cell where the donor DNA is integrated into the recipient DNA.](image)

Antibiotic Resistance

As indicated previously, the primary cause of antibiotic resistance is the transfer of genetically mutated DNA from one bacterial cell to other bacterial cells. The product of all genes is either a structural protein or an enzyme. In this case, the protein from the genetically muted cells acts on the antibiotic to break it down, or in some way inhibit its ability to act on the bacteria.

Based on information about organisms stored/preserved prior to the antibiotic’s discovery, we know that organisms resistant to penicillin were present prior to the discovery of penicillin.

We know that genes for antibiotic resistance develop by natural selection, often in the presence of some environmental stress.

Contrary to popular belief, antibiotic use **does not** produce resistance – it simply screens for resistant bacteria that are already present. Stresses, such as exposure to antibiotics then reveal the antibiotic resistant variant. As non-resistant variants are eliminated, the resistant variant becomes the predominant organism in the environment (e.g. a wound, the GI tract, an abscess, etc.).
Many antibiotic resistant genes rely on plasmids to facilitate their transfer. If a bacterium carries several resistance genes it is called multi-resistant bacteria (MRB) or, informally, a superbug. As resistance becomes more common, a greater need for alternative treatments develops. Despite a push for new antibiotics, there has been a continued decline in the number of newly approved drugs.

**Types of Resistance / Inhibition**

*Inhibition of Cell Wall Synthesis*

With the exception of Mycoplama, all bacteria have cell walls. The synthesis of the cell wall is a perfect target for inhibition of the growth of an organism and the death of the cell. There are several ways this process can occur.

The most common class of cell wall active antibiotics are the β-lactam antibiotics. These would include the penicillins, cephalosporins, cephameics, carbapenemes, monobactams, and β-lactam inhibitors. Additional antibiotics that are not β-lactam inhibitors but inhibit cell wall synthesis in other ways, include vancomycin, and bacitracin, among others.

The cell wall of bacteria is mainly composed of peptidoglycan or a chain of N-acetylglucosamine and N-acetylmuramic acid. These chains are cross-linked with peptide bridges. The result is a fairly rigid structure protecting the bacterium like chain-armor protected soldiers in the middle ages. The enzymes that produce all of these links are referred to as penicillin-binding-proteins (PBPs) because they are the targets for β-lactam antibiotics. When growing bacteria are exposed to β-lactam antibiotics, the antibiotic binds to specific PBPs and inhibits the assembly of the peptodoglycan chains. The result is often the death of the cell.

Bacteria can become resistant to β-lactam antibiotics by three general mechanisms. These are:

1. Prevention of the interaction between antibiotic and the target PBP;
2. Modification of the binding of the antibiotic to the PBP; and
3. Hydrolysis of the antibiotic by β-lactamases.

The first mechanism of resistance is seen only in gram negative bacteria like the *Pseudomonas* species. These organisms have an outer membrane that covers the cell wall. Penetration of the antibiotic into the bacterium requires transit through the outer membrane through pores. Changes in the proteins (porins) composing the pores can result in changes in the size of the pore. The result is a passive exclusion of the antibiotic based on the size of the pore and the greater size of the antibiotic.

Secondly, resistance can occur by modification of the β-lactam antibiotic binding to the PBP. This can be caused by an overproduction of PBP from the acquisition of a new PBP (as in methicillin resistance in *S. aureus*), modification of an existing PBP through recombination (penicillin resistance in *S. pneumonae*), or a point mutation (penicillin resistance in *E. faecium*).

Lastly, bacteria can produce β-lactamases that inactivate β-lactam antibiotics. More than 200
different β-lactamases have been described. Some are specific for penicillins (penicillinases), cephalosporins (cephalosporinases), or carbapenems (carbapenemases). Others have a broad range of activities including inactivating most β-lactam antibiotics.

There are several classifications of β-lactamases. In one of the more simple schemes (Bush Scheme) there are four classes of β-lactamases (A-D). The most common class, Class A β-lactamases, are SHV-1 and TEM-1 penicillinases which have minimal activity against cephalosporins. They are found in gram negative rods such as *E. coli* and *Klebsiella* as well as other organisms such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*.

An issue with these genes (encoding SHV-1 and TEM-1) is that point mutations in specific areas have recently created β-lactamases that have resistance against all penicillins and cephalosporins. When this occurs, these changes result in what are referred to as extended-spectrum β-lactamases (ESBLs). These are especially troublesome because they are transferrable from organism to organism.

Class B β-lactamases are zinc-dependent metalloenzymes that have broad activity against all β-lactam antibiotics, including cephamsycins and carbapenems (with the exception of Aztreonam, which is a monobactam). Currently Class B genes are found mainly in *Pseudomonas aeruginosa* and *Serratia marcescens*.

Class C β-lactamases are primarily cephalosporinases that are encoded on the bacterial chromosome. Usually these genes are repressed from producing the enzymes, but can be “turned on” by being exposed to “inducing” β-lactam antibiotics or mutations in the genes controlling expression of the β-lactamases.

Expression of this Class is especially problematic because they are active against a wide variety of antibiotics including the most potent broad-spectrum cephalosporins. This series of genes is found in many Enterobacteriaceae, including *E. coli*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* (not in the family Enterobacteriaceae). Carbapenems are currently resistant to Class C β-lactamases.

Class D β-lactamases are plasmid mediated enzymes that differ from Class A, but have similar activity. These β-lactamases are active against most penicillins and some cephalosporins, as well as clavulanic acid. This class of β-lactamases are found in gram negative organisms.

![Figure 6: Sites of Action for Antibiotic Activity](image-url)
In 2008, Klebsiella pneumonia was isolated from a patient with an antibiotic resistant infection. The bacterial infection was apparently acquired in New Delhi, although the Indian government disputes this. The isolate was carbapenem resistant along with resistance to a number of other beta-lactam antibiotics. The resistance factor was found to be a novel metallo-beta-lactamase and was designated NDM-1 or New Delhi metallo-beta-lactamase. Subsequently NDM-1 was found in a number of isolates of gram-negative organisms from many sources and areas of the world including the US. This resistance factor is particularly dangerous because of the spectrum of its resistance activity and ease of horizontal gene transfer to other bacteria.

There are additional minor resistance factors that are carried by bacteria that allow resistance to penicillins.

**Vancomycin Inhibition**

Vancomycin is another cell-wall inhibitor that acts effectively on a variety of gram positive organisms. It is too large of a molecule to pass through the outer membrane of gram negative bacteria, thus making them passively resistant to vancomycin.

Some gram positive organisms, such as *Leuconostoc, Lactobacillus*, and *Pediococcus*, are intrinsically resistant. Some enterococci are also intrinsically resistant. *Staphylococcus aureus* (Methicillin susceptible and resistant [MRSA]) have classically been susceptible to vancomycin. Indeed, vancomycin has often been considered the “last resort” in the treatment of MRSA infections.

Strains of *Enterococcus faecium* and *E. faecalis* have acquired a plasmid carrying vancomycin resistance (primarily vanA and vanB). The enzymes cause changes in the cross-bridging of the peptidoglycan making the cell wall resistant to the vancomycin. In and of itself, this is not a major problem. However, conjugation can occur between *Staphylococcus* and *Enterococcus*. The vancomycin resistance plasmid is being transferred to MRSA at a high rate. The plasmid is a multiple-resistance conjugative. The MRSA (already multiple antibiotic resistant) then receives a plasmid that makes it resistant to vancomycin and other antibiotics. The medical implications of this spread of antibiotic resistance is profound.

**Inhibition of Protein Synthesis**

Inhibition of Protein Synthesis includes a number of agents, but most importantly, the aminoglycosides, tetracyclines, and macrolides. Aminoglycosides include the most commonly used gentamicin, tobramycin, and amikacin. Their activity is mainly against gram negative organisms causing systemic infections, although,
there is activity against some specific gram positive organisms.

These antibiotics bind irreversibly to the 30S ribosome. This will act to cause misreadings of the messenger RNA (m-RNA) and interruption of protein synthesis.

Resistance to aminoglycosides occurs in four ways:

1. Enzymatic modification of the antibiotic;
2. Decreased uptake of the antibiotic into the bacterial cell;
3. Increased expulsion of the antibiotic from the cell; and
4. Mutation of the ribosomal binding site.

The first, and most common method is the mechanism of resistance seen in aminoglycoside-resistant bacteria. The modification of the antibiotic is accomplished by the action of phosphotransferases, adenyltransferases, and acetyltransferases on the amino and hydroxyl groups of the antibiotic.

The second method, inhibited transport, has been seen in anaerobes and Pseudomonas.

The third method, active efflux of aminoglycosides, is seen only in gram negative bacteria, but is also rare.

The fourth method can be associated with tetracyclines. Tetracyclines act by inhibiting protein synthesis binding to the 30S ribosome and inhibits transfer RNA (thus differing from aminoglycosides). This antibiotic class is effective against certain specific gram positive and gram negative bacteria, as well as Chlamydia, Mycoplasma, and Rickettsia.

All tetracyclines have similar antimicrobial activity with the major differences being pharmacological. Resistance to these antibiotics are not unlike resistance to aminoglycosides and characteristics would include: decreased uptake, active efflux, alteration of the target on the ribosome, or enzymatic modification of the antibiotic. Most of these resistance mechanisms are due to mutations on the host genes.

Increased efflux is the most common mechanism of resistance seen in bacteria. Macrolides are broad-spectrum polysaccharide-based antibiotics. The classic example is erythromycin. Most commonly used today are clarithromycin and azithromycin. These antibiotics exert their effect by binding to the 23S rRNA of the 50S ribosomal subunit. Resistance most commonly occurs from methylation of 23S rRNA thus inhibiting binding of the antibiotic (see Figure 6).
An example of mutation of the ribosomal binding site is modification of the 23S ribosome or enzymatic destruction of tetracycline, but this is rare.

**Inhibition of Nucleic Acid Synthesis**

Inhibition of nucleic acid synthesis occurs with the quinolones, rifampin, and rifabutin. Quinolones include ciprofloxacin, levofloxacin, consider deleting gati since no longer on market, moxifloxacin, as well as a number of fluoroquinolones. These agents have excellent activity against gram positive and gram negative bacteria. Unfortunately resistance can develop rather quickly in *Pseudomonas*, some staphylococci, and enterococci. Resistance to this class of antibiotics is mediated by chromosomal mutations in the structural gene for DNA gyrase and topoisomerase type IV.

Other mechanisms of resistance include rapid efflux of the antibiotic and decreased uptake of the antibiotic caused by mutations in membrane permeability regulatory genes. Rifampin and a derivative, Rifabutin, are semi-synthetic derivatives that have wide antibiotic activity.

These antibiotics act by binding to DNA-dependent RNA polymerase, thus inhibiting the initiation of RNA synthesis. Unfortunately, as in the previous case, resistance can also develop very rapidly. In gram positive bacteria, resistance can occur by a mutation in the chromosomal gene that codes for a portion of the RNA polymerase. Gram negative bacteria are (or become) intrinsically resistant and do not take up the antibiotic.

**Topics for Further Discussion**

This article was not meant to be an exhaustive treatise of the subject. It is an overview of an extremely complex and important topic. As you may have noticed we did not cover antimetabolites. Most antimetabolites are not strictly antibiotics and we will address these agents in a separate article. Topics of future articles will include the implications of antibiotic resistance in medicine and agriculture. What are archaeocins? We will look at this curious new class of agents. We will also discuss the specific issues related to wound infections, anaerobic infections, and alternatives to antibiotic use, among others.

<table>
<thead>
<tr>
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<th>Producer Organism</th>
<th>Activity</th>
<th>Site of Action</th>
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<tr>
<td>Bacitracin</td>
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<td>Gram-Positive Bacteria</td>
<td>Wall Synthesis</td>
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<tr>
<td>Cephalosporin</td>
<td>Cephalosporium acremonium</td>
<td>Broad Spectrum</td>
<td>Wall Synthesis</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Streptomyces erythreus</td>
<td>Gram-Positive Bacteria</td>
<td>Protein Synthesis</td>
</tr>
<tr>
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<td>Micromonospora purpurea</td>
<td>Broad Spectrum</td>
<td>Protein Synthesis</td>
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<td>Dermatophytic Fungi</td>
<td>Microtubules</td>
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<tr>
<td>Neomycin</td>
<td>Streptomyces fraidae</td>
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<tr>
<td>Penicillin</td>
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<td>Vancomycin</td>
<td>Streptomyces orientalis</td>
<td>Gram-Positive Bacteria</td>
<td>Protein Synthesis</td>
</tr>
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*Figure 8: Inhibition of different antibiotics.*
About Clinical Research Management, Inc.

Clinical Research Management (ClinicalRM) is not only involved in the testing of new antibiotics in Phase I, II, III studies, monitoring protocol development, site selection, and assistance with FDA approvals, but is also involved in responding to the challenges of antimicrobial resistance. Our epidemiologists track resistance patterns around the globe and they evaluate how the observed resistance appears, where it emanates from, and how we can best contain the spread of the new resistance factors.

Our scientists work with the Government and academia to develop new responses to the ever-growing threat of multiple-resistant superbugs. They use in silico techniques, as well as information from genomics, to determine sites on, or in, these organisms that are most likely to be vulnerable to engineered antimicrobials. ClinicalRM is committed to developing new responses to disease and the challenges presented by these super-bugs. If you feel ClinicalRM can add value to your research efforts, we are interested in speaking with you. Call toll free at (800) 431-9640 or visit www.clinicalrm.com

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