Antibiotics: Mechanisms of Action and the Acquisition of Resistance—When Magic Bullets Lose Their Magic

Michel J. Cloutier
Saint Louis College of Pharmacy, 4588 Parkview Place, Saint Louis MO 63110-1088

PROLOGUE
This lecture discusses mechanisms of antibiotic activity as an introduction to a closer look at the diverse mechanisms of innate and acquired, transferable/nontransferable drug resistance. The emergence of multiple-drug-resistant microbes is one of the most rapidly growing challenges facing today’s health care practitioners. The increasingly rapid pace at which microbes are able to develop or acquire new drug-resistance profiles outpaces the rate at which the pharmaceutical industry develops, screens and distributes new antimicrobial agents. This lecture helps students understand, not only the specific mechanisms by which microbes display resistance, but also the forces driving the increasingly rapid rate by which microbes develop, acquire and/or share these mechanisms.

INTRODUCTION
Human members of the biological community of this planet share with all other species the ongoing struggle for survival. From the earliest fragments of recorded history, humans have documented the challenges faced by individuals and societies and our efforts to overcome those challenges. Where extensive documentation exists, clear descriptions of the symptomology of a wide variety of human diseases date back to thousands of years B.C. Among the empirical observations recorded by early healers was one that when healthy individuals spent a prolonged period of time in close proximity to, or came into physical contact with, individuals suffering from certain types of diseases, frequently they too would eventually develop the same disease. It was also observed in the case of some diseases, which were most frequently fatal, that among those individuals who recovered, few if any ever suffered from that same disease a second time. Early medical practitioners eventually developed the concepts of “contagion” and “immunity” and the practices of “quarantine” and “vaccination” from repeated similar observations over the centuries.

That which most intrigues me is the knowledge that these concepts and practices were developed in the complete absence of any accurate information regarding the cause of infectious diseases. The determination of the etiology of infectious disease awaited the development of technology and a shift from the heavy reliance on empirical observations to a careful and purposeful application of the scientific method of investigation. And so it was that thousands of years of guess work eventually gave way to the deliberate experimentations of Louis Pasteur, Robert Koch and others.

In 1857 a French chemist, named Louis Pasteur, was recruited by the government of France to apply his skills to the study of “diseases of the wine” which were plaguing the French wine industry. He was able to demonstrate that the products of fermentation in both good and bad wine were dependent on the types of microorganisms introduced into the grape juice prior to the fermentation process. This represents the first documented cause and effect relationship between specific microorganisms and specific biochemical processes. In 1864 Pasteur presented the results of years of painstaking work with his now famous swan-necked flasks, which quickly proved to be the final blow to the concept of “Spontaneous Generation.” With these discoveries van Leeuwenhoek’s “Animalcules”, which had suffered in relative obscurity for 200 years, rapidly gained new respectability as entities worthy of serious scientific investigation.

In the 1870s a young German physician, Robert Koch, applied his skills to the study of human and animal diseases. As a relatively new scientific discipline, the field of microbiology required the development of laboratory techniques and procedures for the in vitro cultivation of microorganisms. Over the next twenty years, Koch and his students developed a variety of materials and methods to facilitate their work, many of which are still in use today. Although the “Germ Theory of Disease” predated both Pasteur and Koch by some 300 years, the first demonstrated cause and effect relationship between a specific bacterium, *Bacillus anthracis*, and a specific infectious disease, anthrax, was
reported by Robert Koch in 1876. Less than forty years later, the cause and effect relationships between specific bacteria and protozoa and over two dozen major human infections were clearly established. Similar relationships between vi-
uses and diseases followed soon after the invention of the electron microscope and in vitro tissue culture techniques.

Along with these discoveries came major developments in disease prevention such as antiseptic medical procedures, vaccinations, improved sanitation, water purification systems and waste management. Although treatment of disease is almost as old as disease itself, the earliest efforts aimed at specific eradication of the disease causing agent from the patient is credited to Paul Erhlich. In the late 1800s, Erhlich launched a systematic investigation of chemical agents which displayed antimicrobial activity in the labora-
tory for their therapeutic efficacy in the infected patient. Most important among the desired characteristics of these agents were: (i) the ability to quickly and specifically kill the infectious microbe; and (ii) to have no serious ill effects on the human host. For these reasons he referred to them as “Magic Bullets.”

Erhlich’s efforts paid off in 1906 with his discovery of Salvarsan which became the first chemotherapeutic agent in clinical use for the treatment of a specific human infectious disease, syphilis. Advancement in the search for clinically useful antimicrobials was initially slow. Many chemicals with antimicrobial activity proved to be too toxic for clinical use. In 1935, Gerhard Domagk discovered the in vivo anti-
microbial efficacy of a particular red dye called Prontosil. The curious lack of antimicrobial activity in vitro was explained by Jacques Tréfouël and his colleagues in the early 1940s with the discovery that sulfanilamide is liberated when body tissues break down Prontosil. The discovery of sulfanilamide lead to the development of a wide variety of sulfa-drugs some of which are still in use today. It was the discovery of Penicillin by Alexander Fleming in 1928 and its subsequent wide spread clinical application in the 1940s which eventually proved to be the single greatest advance in antimicrobial chemotherapy.

In our haste to take advantage of this most “Magic” of bullets, we inadvertently sowed the seeds of what is rapidly becoming our next great challenge in the control of human infectious disease, the emergence of antibiotic-resistant pathogens. Barely fifty years after the introduction of penicillin, the indiscriminate dispensing of this and many other antimicrobials, has brought us to the threshold of what some have called the “Post Antibiotic Era”(1,2). Old enemies in the battle against infectious disease, once thought to be under control and nearly vanquished, have reemerged with increased vigor and virulence because our “Magic Bullets” seem to have lost their magic(3-5).

MECHANISMS OF ACTION

In spite of its great success, it was obvious from the beginning that some bacteria were not sensitive to penicillin. Although very effective in the treatment of a wide variety of infections caused by Gram-positive organisms, penicillin is limited in its efficacy against Gram-negative organisms. This pattern of inherent drug sensitivity or resistance is characteristic of the specific type of microorganism. Among Gram-negatives, inherent resistance is now known to be primarily associated with the relative impermeability of the structurally complex outer membrane of the bacterial cell wall. And so the search was on to discover new and improved antimicrobials. The next three decades witnessed a rapid increase in the number of broad and narrow spectrum, naturally-occuring, true antibiotics and various synthetic and semi-synthetic antimicrobial agents.

Erhlich’s original concept of “Magic Bullets” lead investigaters to search for unique properties of infectious agents not shared with humans. The structural and functional differences between prokaryotic and eukaryotic cells provide a variety of unique characteristics of bacteria which could be specifically targeted for disruption by chemical agents. Among these targets is the bacterial cell wall. Although cell walls are by no means restricted to prokaryotes, the primary structural component—peptidoglycan—is specific to bacteria. Therefore, each of the many enzymatically catalyzed steps involved in the synthesis of individual components and the final assembly into the macromolecular structure theoretically provides a specific target for chemical intervention. Analysis of microbial membranes, metabolic pathways and the mechanisms of replication, transcription and translation have all revealed unique characteristics with potential for exploitation as targets of chemotherapeutic intervention. Figure 1 illustrates these targets and some of the antibacterial agents which have proved useful in our war against infectious disease.

ANTIBIOTIC RESISTANCE

Soon after the introduction of penicillin, sporadic reports of drug-resistant strains of previously sensitive bacteria began to appear. In hindsight, we see these reports as the early warning signs of trouble in the making; for the most part they were not regarded as significant since the development of resistance had been demonstrated in the laboratory and was easily overcome by increasing the dosage. Eventually the occurrence of drug-resistant strains in the wild became more widespread, and the practice of raising the dosage proved increasingly ineffective. Still, the health care community failed to heed this warning since alternative therapies were
available. The mid-seventies to early-eighties witnessed the arrival of multiple-drug-resistant strains of microbes, and finally, concerned researchers began to sound the warnings. Since the arrival of AIDS on the scene in 1981, we have been forced to deal with a shrinking pharmacopeia as drug after drug has been removed from the list of clinically useful antimicrobials in the face of a seeming explosion of single- and multiple-drug-resistant microbes.

Unlike the earlier challenges posed by inherent resistance, today’s battles are precipitated by the ever-increasing rate of acquired resistance. Although research into the mechanisms of action of antimicrobial agents was usually initiated soon after the discovery of each active agent, significant progress in the research involving antibiotic resistance was slow until major advances in genetics provided the means to investigate and understand the specific mechanisms by which microbes acquire drug resistance.

To understand the threat that microbial acquired drug-resistance poses to the future of human health, we need only to study the reports of the last few decades regarding the rate of the appearance of new and increasingly diverse drug-resistant strains(2). In order to meet and overcome this challenge, we must understand the molecular mechanisms by which microorganisms acquire drug-resistant capabilities as well as the environmental forces driving this accelerated evolution.

GENETIC BASIS OF ACQUIRED RESISTANCE

An understanding of the molecular mechanisms involved in the acquisition of new genetic information, either through mutation or gene transfer, followed on the heels of some major breakthroughs in biochemistry, genetics and molecular biology. In 1944 Avery, MacLeod and McCarty described the process of transformation in bacteria whereby genetic information from dead organisms could be acquired by living organisms. Their research also provided the conclusive evidence that the genetic material was in fact DNA.

Two years later, Lederberg and Tatum described the process of bacterial conjugation in which plasmid-, as well as genomic-, DNA could be transferred from one living bacterium to another. In 1951, while attempting to demonstrate conjugation in species other that Eschericia coli, Lederberg and Zinder discovered the process of transduction, the transfer of bacterial genes by viruses. In 1953 Watson and Crick proposed their model for the structure of DNA. It was one thing to accept the concept that the genetic information lay in the sequence of bases in the DNA and that changes in this specific sequence, mutations, could alter an organism’s phenotype. It was quite another story to understand how the sequence of nucleotides in a strand of DNA could possibly determine the sequence of amino acids in a protein. For the answer to that question, science waited over fifteen years until the genetic code was finally broken by Holley, Khorana and Nirenberg in the late 1960s. This was soon followed with the discovery of restriction enzymes by Arber, Nathans and Smith in 1971, opening the door to the world of genetic engineering.

Generally, the sum total of the genetic information, the genome, which defines the phenotypic characteristics of a bacterial cell is found in a single, double stranded, closed, circular piece of DNA. Some bacteria also carry additional pieces of extragenomic DNA known as plasmids. These plasmids are small pieces of DNA only large enough to carry a limited number of intact genes, but importantly, copies of plasmids can be shared among different bacteria. Genetic variation between strains of a species and even between strains of different bacterial species involves random mutation, various mechanisms of gene transfer, and is driven by the forces of evolution.

Genetic variation mediated by mutation usually involves small changes in the sequence of bases of existing genes. Such a mutation in a structural gene may result in a single amino acid substitution in the protein product of translation. This may result in: (i) no significant effect in this protein’s function; (ii) complete inactivation of the protein; or (iii) if this protein is the target of a specific antibiotic, render it completely insensitive to the drug, producing a resistant organism. Mutations in regulator genes may activate the expression of existing, previously silent genes, coding for resistant variants of the drug target. This type of mutation may also result in the production of enzymes capable of inactivating specific drugs or providing an alternative biochemical pathway not sensitive to the specific drug therapy again conferring drug-resistance.

Although mutations can, and do, produce antibiotic-resistant strains of bacteria, for these organisms to pose a serious hazard to the public health, they must propagate for many generations under the constant selection pressure provided by the presence of the drug until resistant strains become the dominant organism in the environment. The greatest threat comes from those organisms which have the capacity to share genetic information with their neighbors either through transformation, transduction or conjugation. Via these mechanisms, resistant genes may be rapidly propagated throughout a microbial population, sometimes involving many different species, in a relatively short time.

The key to the development of drug-resistant strains of microorganisms lies in the maintenance of the selective pressure in the environment which: (i) permits the development of the resistant strains; and (ii) eliminates the drug-sensitive strains, allowing for the unchecked proliferation of the resistant microbes until resistance becomes the dominant phenotype. Beginning with penicillin in the 1940s, and continuing with a variety of antimicrobials well into the ‘80s and early ’90s, we humans have provided optimal conditions for the forces of natural selection and survival of the fittest for the development and persistence of drug-resistant microbes.

The human, domestic and wild animal populations constitute the largest natural reservoir for both commensal and pathogenic microorganisms. Inappropriate dosing with antibiotics resulted in elevated levels of antibiotics in significant numbers of certain segments of the population. The wide-spread practice of prescribing antibiotics for non-bacterial infections in the belief that “it couldn’t hurt” was tragically shortsighted. The use of antibiotics as feed supplements in agriculturally important food stuff animals, such as cattle, hogs, poultry, and sheep, to prevent economic losses due to the rapid spread of disease in the feed-lot environment provides fertile ground for the development of antibiotic resistance and for the direct transmission of resistant pathogens to humans(6,7).

Prophylactic therapy with antibiotics was, is, and will continue to be appropriate in specific circumstances involving high-risk, compromised patients. The agricultural uses of antibiotics as feed supplements, in aquaculture and in veterinary medicine have all experienced severe review and
MECHANISMS OF ACQUIRED RESISTANCE

The early observation that only some microorganisms were sensitive to specific antimicrobial agents was attributed to intrinsic resistance. Given the increasing number of therapeutically useful agents being developed with divergent spectrums of activity and the apparent stability of drug sensitivity or resistance within a species, resistance was not viewed as a significant problem. It was not until acquired resistance to relatively high doses which could be transferred laterally within a species, and in some cases between different species, that drug-resistance was recognized as a serious threat to the future of human health care.

The hospital environment, with its confined population of compromised patients requiring antibiotic therapy, is well suited for the development of drug-resistant microbes. As a result, nosocomial infections, infections acquired during hospitalization, are frequently associated with the appearance of new drug-resistant strains of microbes. Health care practitioners soon found themselves confronted with multiple-drug-resistant microbes and a decreasing number of therapeutic options. By the mid-70’s, the most frequent cause of nosocomial infections, Staphylococcus aureus, had developed multiple-drug resistance, remaining sensitive only to methicillin and vancomycin. It was then that reports of strains of methicillin-resistant S. aureus (MRSA) began to appear around the world, including the United States (8).

Table 1 lists the basic mechanisms of acquired resistance, some of the agents whose therapeutic efficacy has been compromised, and representative pathogens which have acquired resistance.

Permeability Barriers. From the beginning, it was understood that for an antimicrobial agent to be effective, it first had to gain access to its specific site of action within the microbial cell. Early explanations for the differences in sensitivity and resistance of various microbes to a variety of drugs was largely attributed to the relative permeability, or impermeability, of bacterial cells. The structural complexity of bacterial plasma membranes, walls, outer membranes and/or wax-like mycolic acid bilayers pose formidable permeability barriers. Bacteria acquire all of their essential nutrients from the external environment. Therefore, mechanisms of active or facilitated transport must exist if the cell is to survive. These same transport systems are, in part, responsible for facilitating drug accessibility to the target site. The enormous variety of structural components encountered within the diversity of bacterial species accounts for much of the observed intrinsic resistance to antimicrobial agents exhibited by certain bacterial species.

The enormous number of individuals representing any given species of bacteria provide for tremendous genetic and phenotypic variety within that species. In the absence of selective pressure, this variety within the population will achieve a stable equilibrium. With the introduction of antibiotics, the forces of natural selection provided those naturally occurring variants, less sensitive to the drugs, with a competitive advantage over more sensitive strains. Gradually these “less sensitive” strains became dominant in the population. Early reports of acquired resistance, overcome by increasing the drug dose, were most probably the result of this kind of selection of naturally occurring variants within the population.

Membrane permeability to small molecules is largely mediated by small channels through the lipid bilayer. Mutations in the porin genes, resulting in reduced expression, or activity, of these channels, accounts for much of the observed decrease in membrane permeability. By itself, increased resistance as a result of reduced membrane permeability usually does not result in a significant threat to the agent’s clinical usefulness. Even if the minimal inhibitory concentration (MIC) is increased by 100 percent, decreased permeability is clinically significant only in conjunction with some other resistance mechanism, resulting in a several-fold increase in MIC(9).

Membrane permeability also involves the active transport of molecules out of the cell. These export channels are also subject to evolution, and the appearance of “active efflux” mechanisms, by themselves, have seriously compromised the efficacy of certain drugs. The combination of reduced permeability and active efflux mechanisms in the same microbe produces a synergistic effect, rendering some agents virtually useless and frequently requiring alternative therapy(10,11).

Enzymatic Inactivation. Probably the best-known mechanism of antibiotic resistance is that associated with the acquisition of plasmid-born genes, coding for degradative or modifying enzymes. Hydrolytic enzymes cleave a critical portion of the antibiotic, rendering it ineffective. Penicillinase, one of a class of β-lactamases, is responsible for cleaving the β-lactam ring, converting penicillin to penilloic acid. To overcome this form of resistance, second and third generation semi-synthetic antibiotics were developed to: (i) resist enzymatic degradation; and (ii) enhance or at least retain antibacterial activity. The clinical application of these new drug variants was soon followed by the appearance of hydrolytic enzymes capable of degrading each new drug(12).

Unlike hydrolytic enzymes which degrade the drug, modification enzymes alter the drug frequency by acetylation or methylation. The resultant molecule is structurally different than the parent drug, usually rendering it incapable of binding to its target enzyme. Enzymatic inactivation by either mechanism is very effective in impairing resistance(12,13).

Metabolic Bypass. The antimetabolites, trimethoprim and sulfamethoxazole (TMP/SMX), interfere with the metabolism of folic acid, which is a required component in the biosynthesis of nucleic acids in bacteria. Many bacteria exhibit intrinsic resistance to TMP/SMX, and some are capable of transferring this genetic capacity to others via plasmids. Acquired resistance to these antimicrobial agents is conferred by the acquisition of plasmids carrying these genes. Unlike the situation with plasmid-born, altered target genes, genes for drug resistant dihydropteroate synthetase and dihydrofolate reductase represent unaltered wild type genes from another source. The resultant microbe expresses both drug sensitive and resistant enzymes.

Altered Targets. Each antibiotic has a unique mechanism of action associated with a specific enzymatic process vital to the survival of the bacterial cell. In the same way that substrates bind to enzymes via very specific molecular inter-
Table 1. Antibacterial drug resistance mechanism

<table>
<thead>
<tr>
<th>Permeability Barriers</th>
<th>Representative Mechanisms of resistance</th>
<th>Antimicrobial agents</th>
<th>Representative pathogens</th>
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<tbody>
<tr>
<td>Reduced membrane permeability</td>
<td><strong>β-lactams</strong></td>
<td>Penicillins, Cephalosporins, Monobactams, Carbapenems</td>
<td>Pseudomonads, Enterobacter, Serratia, Klebsiella</td>
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<tr>
<td>Quinolones</td>
<td>Norfl Roxacin, Ofloxacin, Ciprofloxacin, Lomefloxacins</td>
<td>Enterobacteriaceae, Pseudomonads</td>
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<tr>
<td>Aminoglycosides</td>
<td>Gentamicin, Tobramycin, Amikacin</td>
<td>Bacteroides, Pseudomonads, Enterobacteriaceae</td>
<td></td>
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<tr>
<td>TMP/SMX Fosfomycin</td>
<td></td>
<td>Pseudomonads, Campylobacter, Staphylococcus, Serratia</td>
<td></td>
</tr>
<tr>
<td>Active efflux Tetracyclines Tetacycline Minocycline Doxycycline</td>
<td>Staphylococci, Streptococci, Enterobacteriaceae Bacteroides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzymatic Inactivation Hydrolysis</td>
<td><strong>β-lactams</strong></td>
<td>Staphylococci, Enterococci, Pseudomonads, Acinetobacter, Moraxella, Bacteroides, Neisseria, Enterobacteriaceae</td>
<td></td>
</tr>
<tr>
<td>Modification Aminoglycosides Macrolides Erythromycin Clindamycin</td>
<td>Staphylococci, Streptococci, Enterococci, Pseudomonads, Enterobacteriaceae Neisseria</td>
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<tr>
<td>Chloramphenicol</td>
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<tr>
<td>Altered Target</td>
<td><strong>β-lactams</strong></td>
<td>Staphylococci, Streptococci, Haemophilus, Neisseria Ersheria, Pseudomonads, Enterobacteriaceae, Enterococci, Mycoplasma, Ureplasma</td>
<td></td>
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<tr>
<td>Quinolones Aminoglycosides Tetracyclines Rifampin TMP/SMX Glycopeptides</td>
<td>Vancomycin, Teicoplanin</td>
<td>Enterococci, Eeuconostoc, Eactococcus, Pediococcus, Eactobacillus Staphylococci</td>
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<tr>
<td>Mupirocin</td>
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<tr>
<td>Metabolic Bypass TMP/SMX</td>
<td></td>
<td>Staphylococci, Streptococci, Neisseria, Enterobacteriaceae</td>
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</table>

actions, antibiotics also require highly specific binding sites. We have already seen how modification of the drug can block binding to the target site. Alteration of the target can also block binding of the drug.

The antibiotic activity of penicillin is associated with binding to a series of proteins intimately involved in the enzymatic process of peptidoglycan synthesis. Each of these different penicillin binding proteins (PBPS), over half-a-dozen to date, is associated with a specific enzymatic process. It appears that in some cases, binding of penicillin results in non-competitive inhibition of the enzyme since some mutations in the PBPS result in drug-resistance without affecting the protein’s ability to bind penicillin (14).

Target site alterations in DNA Gyrase, DNA-directed RNA-polymerase, ribosomal structural proteins and rRNA’s are responsible for a wide variety of antibiotic resistance profiles. Most of these alterations are the result of genomic mutations producing non-sensitive variants of the original target, but some of these resistance genes are found on plasmids. High level vancomycin resistance is transferable and most probably involves a gene coding for a drug-insensitive target (14,15).

Vancomycin resistance made its first clinical appearance in Enterococci in the late-80s (16). Although transfer of resistance has been demonstrated in the laboratory, transfer of vancomycin resistance in the wild has not yet been documented. Should vancomycin resistance develop in MRSA’s, we will be faced with an infectious agent for which
we presently have no effective therapy. Given the demonstrated propensity of S. aureus to collect antibiotic-resistant plasmids, the acquisition of vancomycin resistance by MRSA’s becomes a question of when, not if(4.5).

CONCLUSION

Interest in research and development of effective antimicrobials declined steadily through the 1970s and mid ’80’s, due in part to the escalating costs associated with the research and testing required to bring new drugs to market and a growing complacency in the belief that our existing armament of antimicrobials was adequate for the treatment and cure of most of the major human infectious diseases. In spite of the fact that reports of drug-resistant strains of infectious agents previously susceptible to penicillin began to appear in the first decade following its introduction as an antifungal agent, the medical community and the pharmaceutical industry continued to believe that the existing alternative drugs would be sufficient to deal with these rare and presumably transient microbes.

Major advances in municipal waste management and water treatment, as well as advances in public health and medicine including antimicrobial chemotherapy occurred around the turn of the century. All contributed to a considerable increase in human life expectancy. Along with advanced age comes an increase in the kinds and duration of organic, non-infectious diseases and a gradual decrease in innate resistance and immune competency. Also immunosuppression, either deliberate as in the case of tissue and organ transplantation, as a consequence of anticancer chemotherapy, or as a result of HIV infection, has provided a growing population of compromised hosts with increased susceptibility to infection. These individuals provide fertile ground for microorganisms to thrive and share genetic information which may lead to the development of new drug-resistant strains.

We are beginning to pay the price for our early cavalier approach with regard to the widespread indiscriminate use of antibiotics in health care, agriculture and veterinary science. In our zest to cure infections and improve the human condition, we lost sight of the fact that microorganisms are also subject to the rules of Darwinian evolution. Natural selection worked on the Galapagos finches, like wise microbes will continue to change over time in response to the environmental pressures imposed by elevated levels of antimicrobial agents.

In the past fifty years we have seen how survival of the fittest has favored the gradual appearance and recent explosion in the number and nature of antibiotic-resistant microbes. If we are to keep from returning to the days where the absence of effective chemotherapy meant that infectious diseases were the number one cause of death, we must not only sustain our recently renewed efforts in anti-microbial research and development but also remember the lessons learned and utilize our newly developed “Magic Bullets” with new-found discretion.


References


APPENDIX. BIBLIOGRAPHY

The reader is referred to the following for further study:


Travis, J., “Reviving the antibiotic miracle?”, *Science*, 264, 360-362 (15, April 1994).


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