Development of Antimicrobial Agents in the Era of New and Reemerging Infectious Diseases and Increasing Antibiotic Resistance

Gail H. Cassell, PhD
John Mekalanos, PhD

Magnitude of the Problem
Infectious diseases are one of the leading causes of death worldwide, accounting for 13.3 million deaths (25% of all deaths) in 1998. Five of the 10 US leading causes of death are related to infectious diseases (pneumonia, acquired immunodeficiency syndrome [AIDS], chronic liver disease, chronic obstructive lung disease, and cancer). Between 1980 and 1992, the US death rate from infectious diseases increased 58% (including only people for whom the primary cause of death was an infectious disease). The increase was not only due to deaths associated with AIDS but to pneumonia and sepsis in other patient populations. Furthermore, the incidence of acute otitis media increased 64% among children between 1982 and 1991, and otitis media diagnosis was the most commonly recorded emergency department diagnosis in 1992. Antibiotics are now the second most commonly prescribed category of drugs.

The reasons for the increase in incidence of infectious diseases are not fully understood. Changes in human demographics and behavior (eg, increasing use of day care facilities, a risk factor for otitis media); technology and industry; economic development and land use (possibly accounting for an increase in zoonotic diseases); international travel and commerce; and breakdown of public health measures are thought to contribute to new infectious diseases and re-emergence of infectious diseases thought to have been controlled. For example, tuberculosis, malaria, and cholera have reemerged or spread geographically since 1973, often in more virulent forms. More than 30 new disease agents have been identified since 1973. Escherichia coli 0157, first identified in humans in the 1980s, has caused numerous disease outbreaks and deaths associated with contaminated food and water. The doubling of US food imports during the last 5 years contributes to millions of foodborne illnesses and deaths, many of which are due to newly identified pathogens. In 1993, a new hantavirus caused deaths associated with a pulmonary syndrome in the southwestern United States. In 1997, an avian strain of influenza began to kill previously healthy people in Hong Kong. In 1999, the first known human case of West Nile virus infection...
was recorded in the western hemisphere. In 2000, a new hemorrhagic fever virus, the Whitewater Arroyo virus, caused deaths in California.

The infectious disease crisis in the United States has resulted in 2 presidential directives, a 1996 directive calling for a more focused infectious diseases policy, particularly for disease surveillance, and a subsequent directive on food safety. An interagency task force was established by the Office of Science and Technology Policy to address the global health threat from infectious diseases. The impact of new infectious agents and the devastating economic impact of AIDS, tuberculosis, and malaria have been high on the agenda of international economic summits for the past 4 years. The US State Department’s Strategic Plan for International Affairs includes protecting human health and reducing the spread of infectious diseases as US strategic goals, and the secretary of state announced in December 1999 the second of 2 major US initiatives to combat human immunodeficiency virus (HIV)/AIDS. The interest of the United Nations in the threat to Africa from HIV/AIDS reflects the international community’s concern about the threat of infectious disease.

Antibiotic Resistance

The danger of new and reemerging infections is compounded by the increase in antibiotic-resistant bacteria. Drug options for treatment of infections are increasingly limited and, in some cases, nonexistent. Although defining the precise public health risk of emergent antibiotic resistance is not simple, there is little doubt the problem is global in scope and very serious.

The economic impact of antimicrobial resistance is substantial. The estimated annual cost of antimicrobial resistance in hospitals due to *Staphylococcus aureus* is $122 million and of nosocomial infections is $4.5 billion. In 1992, 19000 deaths were directly caused by nosocomial infections, the 11th leading cause of death in the United States, and in intensive care units, 28% of nosocomial infections were resistant to the preferred antibiotic treatment. More than 90% of strains of *S aureus* in US hospitals are resistant to penicillin and β-lactam antibiotics, and the incidence of vancomycin-resistant enterococci in the United States increased 20-fold from January 1989 to March 1993. Entero- cocci are the most common cause of nosocomial infections, and vancomycin is often the only effective agent.

Vancomycin was previously the most effective drug for methicillin-resistant *S aureus*. However, in 1997, strains of *S aureus* with decreased susceptibility to vancomycin were reported in Japan and the United States and subsequently have been reported in at least 2 other countries. In the hospital environment, gram-negative bacteria are increasingly resistant to extended-spectrum cephalosporins.

Community-acquired antibiotic resistance is also a growing concern. Before 1987, antibiotic-resistant *Streptococcus pneumoniae* (pneumococci) were uncommon, but in some communities up to 40% of strains are now resistant to penicillin. Between 1993 and 1997, the frequency of penicillin resistance increased from 14% to 25%. Pneumococci are the leading cause of pneumonia, meningitis, and bloodstream infections in elderly persons and of otitis media in children. The increase in antimicrobial resistance has led to emergence of strains that are susceptible only to vancomycin. Many other pathogens, including the agents that cause malaria, tuberculosis, gonorrhea, and salmonella, are becoming resistant to standard therapy.

The complacency associated with infectious diseases in the 1960s and the general confidence in existing antibiotics resulted in a lag in production of new classes of antimicrobial agents, despite advances in the fundamental science that has stimulated pharmaceutical innovation in other areas.

Antibiotics and Natural Products

Until approval of an oxazolidone by the FDA this year, no new class of antibacterial drug had been approved for more than 25 years. During this period, the pharmaceutical industry pursued chemical modification of existing classes of drugs, most of which are natural products of bacteria and fungi. This approach provided relief from the resistance crisis, but its impact has been limited. Modified versions of old drugs may be ineffective because existing resistance mechanisms may either be partially cross-effective or mutate to broader specificities that encompass the newer derivatives. Because most antibacterial agents are natural products, resistance mechanisms for these have undoubtedly coevolved with the organisms that have produced these compounds over millions of years. Indeed, the genes encoding resistance to antibiotics are often present within the actual organism that produces the antibiotic and, thus, need only to move from soil organisms to pathogenic microbes for resistance to emerge. Because of this relatively rapid mechanism of acquiring resistance, most antibiotics derived from natural products will have only a limited period of practical clinical utility.

Antibiotics From Xenobiotics

A theoretical way to extend the effectiveness of antimicrobial compounds would be to base them on nonnatural compounds that are chemically foreign to the biosphere (ie, xenobiotics). If no microbe has ever been exposed to an antibiotic or compounds structurally related to it, then the chance that enzymes already exist in nature that can destroy this drug would be lower. Consequently, emergence of resistance by horizontal genetic exchange would be less likely.

Faced with such “xenoantibiotics,” bacteria would need to either mutate the target of the drug (an event which might be simple or might not occur, depending on how the drug works) or use a cross-resistance mechanism such as efflux. Drug efflux systems can be specific (eg, those involved in tetracycline resistance) or broad and involve common compounds that have physical-chemical properties such as size, hydrophobicity, and net charge. While many xenobiotic compounds are substrates for efflux, others might not be recognized by any of the efflux pumps currently ex-
tant. Insights from bioinformatics indicate that many drug efflux transporters are evolutionarily related regardless of their specificity. All efflux systems are derived from a common ancestral system; therefore, these proteins may have evolved under the selection pressure of a limited spectrum of toxic natural products. Thus, by steering away from natural products that have coevolved with these efflux pumps, new classes of chemical compounds might be discovered that will not be substrates for the efflux systems currently extant in the biosphere.

**Advances in Synthetic Chemistry**

Although the potential complexity of synthetic and natural products is theoretically infinite, limits in the methods for organic synthesis have made it difficult to compete with nature. However, many believe that the new age of antibiologic discovery will be driven by revolutionary developments in chemistry, genetics, structural biology, bioinformatics, and engineering. Synthetic chemistry has undergone a dramatic transformation in the last decade, driven primarily by combinatorial chemistry. Rather than being limited to “one vessel, one reaction,” as in most serial synthetic chemistry, combinatorial chemistry is characterized by exponential increases in the number of compounds that can be synthesized in each reiterative cycle of reactions. Huge multimillion-member libraries of compounds can be generated without the costs of traditional synthetic chemistry.

Moreover, synthetic scaffolds now make possible the production of chemical libraries of unprecedented molecular complexity and stereochemical diversity. Some polycyclic, extensively functionalized molecules appear as structurally complex as many natural products. The molecular diversity present can theoretically be tapped to find inhibitors of virtually any target protein, given sufficiently sensitive and robust screening protocols. Advances in assay automation, robotics, imaging, and miniaturization (eg, nanotechnology, microarraying methods) are revolutionizing how active molecules are discovered. Merging of advances in high-throughput screening with combinatorial chemistry offers opportunities for formulating new paradigms for antibiotic development during the next decade.

**Genomics and Bioinformatics**

The bacterial genome project provides an unprecedented opportunity to view the entire genetic blueprints of bacterial organisms, beginning first with *Haemophilus influenzae*. More than 30 bacterial genomes have been sequenced, and other microbial genome sequencing projects are in various stages of completion. However, identifying good antimicrobial drug targets on the basis of a genomic sequence is not trivial. Bioinformatics will help identify homologs of known genes by relying on algorithms that detect similarities in sequence or the presence of a diagnostic sequence motif, such as conserved residues in an enzyme’s known active site. Several powerful antibiotics inhibit multiple targets in bacterial cells: quinolones inhibit both DNA gyrase and topoisomerases, and β-lactams inhibit multiple penicillin-binding proteins. Accordingly, finding common motifs in essential proteins might provide a means of selecting unknown target families of enzymes that have no defined biochemical function yet. Algorithms and motif searches that accurately predict localization and membrane topology of proteins might aid in the search for better potential drug targets. An essential gene product that is localized in more accessible parts of the cell (eg, on the outer surface of the cytoplasmic membrane) might be easier to inhibit because drugs would not need to penetrate into the cell and, therefore, would not be as susceptible to cytoplasmic modification, degradation, or efflux resistance mechanisms.

Comparison of bacterial target genes with human genes will also be important because a good antimicrobial drug target should have no homologs in mammalian cells (thus minimizing the chances that a drug will cause adverse effects). Although bioinformatics is a powerful tool for formulating hypotheses about gene function, conservation of a gene product between multiple bacterial species is not a guarantee that the gene product is essential for growth and, therefore, a potential drug target. Similar gene products can perform different functions for the cell, and proteins of nearly completely different sequence can perform the same function. The presence of a gene says nothing about when or if it is expressed or how important it is in the biology of the organism. These issues must be considered in formulating drug development strategies based on bacterial genomic sequence.

**Bacterial Functional Genomics**

After the genome of an organism is sequenced, many questions still hamper drug discovery. Typically, 30% to 50% of the several thousand genes that make up each bacterial genome as yet have no apparent function. Many of these unknown genes probably encode valid antibiotic targets, but evolution has selected a limited number of gene products as targets for natural-product antibiotics. One explanation of why natural antibiotics target only 20 or so gene products is that evolution allowed the selection of targets that could be inhibited efficiently in competitive microorganisms but not so efficiently that the antibiotic-producing organism could not devise an intrinsic resistance mechanism against its own drug product. Natural antibiotics may not exist for hundreds of other potential targets because these gene products represent “forbidden fruit” that antibiotic-producing organisms have avoided targeting throughout evolution because their inhibition would also have deleterious effects on their own viability. Thus, valid targets for future generations of antibacterial drugs should include proteins that are known targets of existing drugs and essential genes that are not targets of a natural antibiotic.

With the exception of antibiotics that have detergentlike activity, all known antibiotics inhibit essential genes. From the perspective of finding the most appropriate drug targets of the future, it is desirable to know which bacterial gene products are essential for growth or viability. Methods to facilitate identifica-
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The most important bacterial and fungal pathogens should aid rational drug design against infectious agents. For example, DNA chip or microarray technology will allow understanding of gene expression patterns during infection for bacterial and target host cells. Understanding changes in gene expression within the host during infection might provide ways to identify immunomodulatory molecules important to pathological processes. As new drugs are developed, profiling of gene expression in host cells can be used to understand whether an antibacterial lead compound is likely to have adverse effects on eukaryotic cells and the host.

Similarly, expression of many bacterial virulence properties is regulated by environmental signals associated specifically with host tissues or cells. It will be important to know why each particular regulatory strategy exists, the specific virulence genes that respond to the regulation, the environmental signals in the host that are involved, and how to use this information to combat pathogenic microbes through development of novel therapeutic agents or antimicrobial drugs. Transcription profiling can also be used to monitor bacterial changes during stresses such as exposure to antibiotics or host defense mechanisms (eg, oxidative stress, cell wall attack by complement). In this way, new targets might be identified to sensitize bacteria to the effects of otherwise less potent drugs.

The biological, immunological, and physiological consequences of the interaction of pathogenic microbes with host have begun to be appreciated during the last decade and provide opportunities to develop new therapeutic options. These new approaches will synergize with antibiotic use in ways that will lead to better clearance of infectious organisms from tissues and better tolerance of their pharmacologically active by-products.

### Prospects for the Future

New and emerging infectious diseases and the potential of bioterrorist attack will pose a rising global health threat and will complicate US and global security for the next 20 years. The future impact of infectious diseases will be influenced by 3 variables. The first is the relationship between increasing microbial resistance and success in developing new antibiotics and vaccines. The second is the trajectory of developing and transnational economies, especially the basic quality of life of the poorest groups in these countries. The third is the degree of success of global and national efforts to create effective systems of surveillance and response. Moreover, the idea that infectious disease will continue to decrease in the United States and other developed countries and be replaced in time by noninfectious causes of death requires reconsideration. Both the increasing impact of infectious diseases on mortality and current understanding of the
processes that affect infectious disease trends need to be taken into account. The prediction that the infectious disease threat may not diminish is supported by evidence that infectious agents cause or contribute to many cancers and chronic diseases previously thought to be caused by environmental or lifestyle factors. The era of molecular biology and intensive research efforts in AIDS have led to sensitive technology for detection of infectious agents. These diagnostic tools and the realization that organisms of otherwise unimpressive virulence can produce slowly progressive chronic disease with diverse clinical manifestations and outcomes have resulted in new concepts of infectious diseases. The demonstration that the final outcome of infection is as much determined by the genetic background of the patient as by the genetic makeup of the infecting agent is rapidly leading to a belief that several chronic diseases of humans of unknown etiology are caused by 1 or more infectious agents. For instance, peptic ulcers are due to Helicobacter pylori, and at least 1 form of chronic arthritis and brain disorders can be caused by Borrelia burgdorferi, most likely through induction of autoimmunity. Recent data obtained in humans and animal models also suggest that mycoplasmas may cause some cases of chronic lung disease in neonates and chronic asthma in adults. Chlamydia pneumoniae recently identified as a common cause of acute respiratory infection, has been shown to be significantly associated with atherosclerosis. Experimental in vitro and animal studies and recent antibiotic treatment trials suggest that its association is causal. In addition, infectious agents cause or contribute to neoplastic diseases in humans, with estimates that as much as 15% of cancers could be avoided by preventing the infectious diseases associated with them, including more than 50% of stomach cancers (due to H pylori) and cervical cancers (due to human papillomavirus) and 80% of liver cancers (due to hepatitis B and C). In contrast with many infectious agents, the basic biology of organisms implicated in chronic diseases and cancer is relatively unknown. With rare exceptions, the means by which pathogens suppress, subvert, or evade host defenses and establish chronic or latent infection have received little attention. Few areas of basic research compared with microbial latency hold greater promise of contributing to the understanding of infectious diseases and the eventual relief of human illness. Given that many of these diseases are so common, the impact on reducing health care costs could be substantial, even if only some cases are proven to be of infectious origin and effective therapies and vaccines can be developed. Thus, research to clarify the etiologic agents and pathogenic mechanisms involved in chronic diseases and cancer deserves the highest priority, especially through application of functional genomics and integrative biological technologies.

In this article we will discuss major categories of infectious disease that threaten human health today. As we consider each category, we will point out the biological mechanisms that contribute to disease pathogenesis, the molecular basis for pathogenesis, emerging trends, and new research approaches that are providing insights into disease mechanisms. The knowledge we gain through these approaches will allow us to identify new targets for vaccine and treatment development.

References