The Research Agenda of the National Institute of Allergy and Infectious Diseases for Antimicrobial Resistance

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(See the article by Dagan et al., on pages 1094–102; the editorial commentary by Rice, on pages 1079–81; and the editorial commentary by Friedman and Whitney, on pages 1082–3.)

Antimicrobial resistance is an intrinsic and inevitable aspect of microbial survival that continually challenges human health. Research on antimicrobial resistance is central to the mission of the National Institute of Allergy and Infectious Diseases (NIAID). In fiscal year 2007, NIAID invested more than $800 million to support basic and translational research on antimicrobials, more than $200 million of which is devoted to understanding the causes, consequences, and treatments of antimicrobial drug resistance. The complex process that facilitates the transformation of ideas into therapies requires a pipeline that runs from bench to bedside, and NIAID has leveraged the entire spectrum of conventional and biodefense resources. NIAID works in partnership with other federal agencies, industry, foundation partners, and foreign governments. The basic and clinical research supported by NIAID will, ideally, continue to yield profound rewards in terms of the understanding, diagnosis, and treatment of infectious diseases.

Antimicrobial resistance occurs in viruses, bacteria, fungi, and parasites as a natural and unavoidable manifestation of their evolutionary capabilities. The decline in effectiveness of existing drugs is a consequence of a complex interaction among natural selection, environment, and patterns of drug use and misuse. As a result, antimicrobial resistance has developed into a global public health issue, as strains of a variety of pathogens have recently emerged that defy treatment with commonly available therapeutics.

In 2005, an estimated 94,360 individuals in the United States developed an invasive infection with methicillin-resistant Staphylococcus aureus (MRSA), and 18,650 patients died [1]. Treatment of patients in intensive care units in the United States has become increasingly complicated by rising rates of multidrug resistance associated with Pseudomonas aeruginosa bloodstream infections, up from 4% in 1993 to 14% in 2002 [2]. Chloroquine-resistant malarial strains have contributed to the resurgence of malaria throughout the world, and reports of resistance to second-line artemisinin-based therapies have already surfaced [3]. In 2004, according to a World Health Organization estimate, 424,203 cases of tuberculosis (TB) were multidrug resistant (MDR-TB) [4]. A 2001–2004 survey of an international network of TB laboratories found that ~20% of all TB cases worldwide were MDR-TB, and ~10% of all MDR-TB cases were extensively drug resistant (XDR-TB), with resistance to both first- and second-line anti-TB drugs [5, 6]. In an outbreak in South Africa at Tugela Ferry, KwaZuluNatal, XDR-TB was diagnosed in 266 patients, and, at one of the town’s hospitals, 85% of the patients being treated died [7, 8]. CLEARLY, a better understanding of how drug resistance arises and how it can be prevented or managed is needed. Recognizing, responding to, and circumventing the processes that contribute to antimicrobial drug resistance are major goals for the National Institute of Allergy and Infectious Diseases (NIAID), which leads the efforts of the National Institutes of Health (NIH) in combating antimicrobial resistance.

Resistance is typically acquired by gene mutation or lateral gene transfer within or between species. Genetic diversity within populations combined with rapid microbial generation time gives microbes a remarkable adaptability in response to selective pressure from antimicrobials. The pool of preexisting resistance genes available in the microbial world is substantial. Recently, genomic analysis of 480 isolates of spore-producing bacteria revealed resistance to 21 different antibiotics, with every isolate being resistant to 7...
or 8 drugs, on average [9]. Because there is no shortage of reservoirs of resistance genes in microbes dwelling in and on humans and environmental surfaces, and given the prevalence and transferability of resistance genes, it is clear that the contest between microbes and antimicrobials will be perpetual.

Many factors exacerbate the growing problem of antimicrobial resistance, including

- microbial population density in health care facilities, which allows transfer of microbes within a community and enables resistance to emerge;
- medical procedures that carry risks of infection with resistant pathogens;
- breakdown in hygienic measures, especially hand hygiene;
- prescribing practices outside approved guidelines for the treatment of infectious diseases;
- lack of rapid, effective diagnostic tests to identify the infecting agent(s) and their antimicrobial susceptibilities.

**NIAID RESEARCH AGENDA**

Funding of antimicrobial research by NIAID has grown steadily over the past decade (figure 1) and currently exceeds $700 million annually. To put the NIAID research portfolio in antimicrobial resistance in perspective, we highlight efforts in 3 critical areas: basic research, translational research, and partnerships. We also address certain representative bacterial diseases affected by the emergence of resistance and the efforts of NIAID to combat these growing problems.

**Basic research.** NIAID supports basic research on pathogens, host-pathogen interactions, mechanisms of drug resistance, and identification of new antimicrobial targets and therapeutics. In recent years, the rate of licensing of new antibacterials has drastically decreased, in part because initial discoveries leading to commercially available antibiotics were based largely on natural products and their analogues developed through medicinal chemistry. Most currently licensed antibiotics are broad spectrum; however, they are derived from only a few chemical classes [10]. Therefore, the quest for genuinely novel antimicrobials relies on the development and exploitation of new strategies. The following examples highlight some of the exciting new approaches being sponsored by NIAID.

NIAID-supported investigators have used microbial genomic sequences to construct in silico metabolic networks in order to identify key enzymes that could serve as potential antimicrobial targets [11]. Virtual chemical libraries based on known reactions are now being generated and tested in silico for their potential to block the active sites of target enzymes. These in silico methods allow for high-volume screening of compounds even before they have been synthesized. Only those compounds found to have the most potential through these virtual methods are synthesized and tested in vitro. Compounds found to be active are further explored by medicinal chemistry to identify those suitable for drug development.

Antibiotics that are currently used only topically because of toxicity may have systemic applications if their toxicities can be ameliorated. However, the costs of synthesizing compounds in an attempt to create less toxic derivatives can be prohibitive. For example, bacitracin is an effective antimicrobial used only topically because of nephrotoxicity. Under an NIAID-supported small-business project, a chemoenzymatic synthesis strategy is being developed that will reduce the cost of synthesizing bacitracin and allow for the development of less toxic derivatives that have the potential for systemic use.

Potential targets for new therapeutics have come from NIH-supported basic research on mechanisms of resistance. Some of these targets and their underlying technology have been licensed for further development (table 1). For example, tetracycline resistance is mediated by an efflux pump that pumps tetracycline from the cell [12]. Basic research determined the crystal structure of the protein pump and discovered the genes that encode these proteins. Armed with this knowledge, companies have developed agents that block the pump. An effective therapy aimed at circumventing tetracycline resistance will include not only tetracycline but also an agent that blocks the efflux pump.

![Figure 1. National Institute of Allergy and Infectious Diseases research funds for antimicrobial research. Bars reflect total dollars awarded in grants, cooperative agreements, contracts, and intramural research on antimicrobial research. This includes, but is not limited to, studies of mechanisms of resistance, identification of new drug targets, identification of new drugs, spread of resistance genes and resistant organisms, clinical studies, and trials of the more efficacious use of antimicrobials.](image)
could prevent the formation of biofilms. For example, researchers have shown that the chelating agent EDTA prevents the formation of biofilms in order to learn how to prevent or disrupt their formation. In recent years, researchers have shown that the chelating agent EDTA prevents the formation of biofilms by Pseudomonas organisms [14]. Other studies have found that lysogenic phages could prevent Pseudomonas organisms from forming biofilms under all physiological conditions tested. These findings could point to specific genes required for biofilm formation and identify targets for drug development.

A critical factor complicating the use of antimicrobials is that their very use creates selection pressure for resistance. This selection pressure can be reduced by limiting exposure of normal microbial flora to the antibiotic. Several NIH-supported research projects focus on photodynamic delivery of drugs to treat microbial infections and cancer. In this drug delivery strategy, when compounds that are nontoxic to microorganisms are illuminated in the presence of oxygen, they generate compounds that are toxic to gram-negative and gram-positive bacteria as well as fungi [15, 16]. By specifically illuminating the site of infection, compounds that are toxic to microorganisms are produced only where they are needed. This technology has the potential to limit selection pressure and reduce the adverse effects of antimicrobial therapy on the patient.

Understanding the pathogenic mechanisms used by a microbe can lead to strategies to defend against it. In recent years, there has been a markedly increased rate of infections caused by community-acquired MRSA (CA-MRSA) [1]. The cytotoxin Panton-Valentine leukocidin (PVL) has been linked epidemiologically to strains of CA-MRSA and is therefore believed to be a major virulence factor. However, NIAID-supported research has demonstrated that PVL is not a virulence factor in mouse sepsis and abscess models [17] but that it may play a role in necrotizing pneumonia [18]. NIAID investigators have shown that virulence does depend on phenol-soluble modulin–like peptides [19]. NIAID-funded research on mechanisms of how MRSA is spread in the household could lead to ways to limit the spread and prevalence of CA-MRSA.

NIAID supports basic research worldwide, including biological resource centers and genomic sequencing services. Biological resource centers offer the research community a common and reliable source of a wide range of strains of microbes and reagents. Since 2000, NIAID has supported a resource center for S. aureus that is particularly useful for studies of antibiotic resistance [20]. NIAID’s extensive genomics program has sequenced >40 bacterial pathogens that infect humans; for many of these, multiple strains have been sequenced [21]. Sequenced pathogens include but are not limited to Enterococcus faecalis, Neisseria

### Table 1. National Institutes of Health (NIH)–funded basic research moved into product development.

<table>
<thead>
<tr>
<th>Products in development</th>
<th>Stage of development</th>
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<tr>
<td>Efflux pump inhibitors that restore potency of antibiotics against drug-resistant gram-negative organisms</td>
<td>Preclinical</td>
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<tr>
<td>Broad-spectrum tetracycline-class antibiotics</td>
<td>Phase 2 clinical study under way for both oral and intravenous formulations in patients with complicated skin and skin structure infections</td>
</tr>
<tr>
<td>Broad-spectrum macrolide antibiotic</td>
<td>Phase 2 clinical trial for treatment of community-acquired pneumonia</td>
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<tr>
<td>Ribosome inhibitor</td>
<td>Phase 1 trials of first candidate initiated in December 2005</td>
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<tr>
<td>Small molecule inhibitors of the MAR proteins that confer resistance</td>
<td>Proof of concept in Escherichia coli, Yersinia, and Pseudomonas by use of clinically relevant models of infection</td>
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<tr>
<td>Novel antibacterial drugs</td>
<td>Lead identification/optimization stage</td>
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<tr>
<td>TB drug that inhibits biosynthesis of the waxy outer coating of the tubercle bacillus</td>
<td>Preclinical studies (2005); entering phase 1 clinical trials</td>
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<tr>
<td>TB drug (nitromidazole) that inhibits protein and cell wall lipid synthesis</td>
<td>Phase 2 clinical trials</td>
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<td>TB drug that inhibits cell wall synthesis</td>
<td>Phase 1 clinical trials</td>
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**NOTE.** MAR, multiple adaptational response; TB, tuberculosis.
Antimicrobial resistance is a growing concern due to the increasing prevalence of pathogenic bacteria that have developed resistance to multiple classes of antibiotics. This resistance can be due to the natural evolution of pathogens or the deliberate introduction of resistance traits into strains of bacteria. The development of new antimicrobial agents is crucial for combating these pathogens, but the process is complex and requires significant resources.

Figure 2. National Institute of Allergy and Infectious Diseases (NIAID) research resources for addressing antimicrobial resistance. The process of developing therapeutically useful products encompasses a range of activities from basic research (blue), through translational research, to the production of new interventions (red). NIAID provides resources and supports research activities at multiple steps along this pathway to translate basic science findings into vaccines, drugs, and diagnostics. GMP, good manufacturing practice; PK/PD, pharmacokinetic/pharmacodynamic.

Translational and applied research. With the dwindling number of resource-rich pharmaceutical companies involved in antimicrobial development, NIAID has helped engage smaller companies and academic investigators who are working to identify new leads for vaccines, therapeutics, and diagnostics. In this regard, NIAID has provided a range of “translational” resources to allow the efficient progression from a basic research concept to a product (figure 2).

Exposure to resistant pathogens is a natural occurrence, but there is also the potential threat of exposure to select agents that have been deliberately engineered to be resistant. Therefore, to reduce the time and cost of creating new antimicrobial products, NIAID has turned to the existing biodefense infrastructure and resources. These include resources for in vitro screening of lead compounds against select agents and resistant strains of important human pathogens and the development and use of animal models. In addition, NIAID’s Vaccine and Treatment Evaluation Units (VTEUs) have facilitated efforts to develop new and improved vaccines and therapies against infectious agents, such as antimicrobial-resistant pathogens. The VTEUs can rapidly enroll large numbers of volunteers into clinical trials aimed at producing meaningful results expeditiously.

NIAID also recognizes that knowledge gaps, particularly in the area of diagnostics, perpetuate the improper use of antimicrobials. It is critical to advance the technology to slow the development of antimicrobial resistance resulting from the inappropriate use of antimicrobial agents. For example, the lack of rapid, sensitive, and specific diagnostic tests for invasive bacterial infections drives unnecessary and inappropriate antimicrobial use. NIAID supports the development of diagnostics for bacterial infections that are or are likely to become antimicrobial resistant. These pathogens include those that cause health care–associated infections (Clostridium difficile, Pseudomonas, Acinetobacter, Enterobacter, Klebsiella, Serratia, Proteus, or Stenotrophomonas [Pseudomonas] maltophilia), bacteremia, candidemia, and community-acquired pneumonia. Through these targeted initiatives and numerous investigator-initiated awards, researchers apply state-of-the-art technologies to identify bacterial pathogens and their products in clinical materials.

Proper dosage is critical for balancing the effectiveness of a drug with its toxicity and can limit the development of antimicrobial resistance. For many antibiotics in use today, detailed pharmacokinetic and pharmacodynamic (PK/PD) information is lacking. For instance, use of the antibiotic colistin was discontinued because of neuro- and nephrotoxicities. However, colistin is needed when persons are infected with bacteria resistant to all frontline classes of antibiotics [22]. Because colistin was first approved for use by the Food and Drug Administration (FDA) when detailed PK/PD data were not required, NIAID is currently supporting a study to obtain these data, particularly in patients being treated with hemodialysis or related technologies. In addition, NIAID has requested proposals that apply PK/PD principles to studies of preventing the emergence of antimicrobial resistance.

Creating effective vaccines against bacterial pathogens would also reduce the occurrence of antimicrobial resistance and alleviate the need for new antimicrobials. Vaccines against S. pneumoniae in the United States have reduced the rate of invasive pneumococcal disease among vaccinated children <5 years of age by 94% [23]. A similarly effective vaccine against staphylococcal infections would reduce the need for new antimicrobials. Four different groups of NIAID-supported researchers have been working on candidate staphylococcus vaccines, including surface proteins [24, 25] and surface polysaccharides [26], all of which have been shown to be protective against staphylococcal infection in animal models. Academic and government researchers have become partners in this endeavor, and NIAID will continue to encourage and support their efforts.

Effective treatment strategies for existing and emerging infectious diseases are needed to limit the development of antimicrobial resistance. Well-designed and executed clinical trials are crucial to the
rational use of antimicrobials. Clinical trials need to address standard-of-care antimicrobial treatment versus shorter durations of therapy or no antimicrobial therapy at all. Because pharmaceutical companies have little incentive to conduct clinical trials using generic drugs, NIAID plays a key role in ensuring that the value of each potentially active drug is fully realized by organizing and supporting clinical trials (table 2). If generic antimicrobials can be inexpensive and effective front-line agents, newer antimicrobials can be held in reserve.

Antimicrobial resistance often arises from the inappropriate use or misuse of antibiotics. Although antibiotics are still commonly prescribed to children with acute otitis media (AOM), we await a placebo-controlled clinical trial evaluating the effectiveness of antimicrobials for treating AOM. NIAID is currently sponsoring a clinical trial to determine the efficacy of antimicrobials in young children with AOM. The time to resolution of symptoms in young children diagnosed with AOM receiving antimicrobial therapy will be determined and compared with that in children receiving placebo. These data will guide the decision making for physicians treating young children with AOM, thus reducing the risk of antimicrobial resistance.

Skin and soft-tissue infection caused by CA-MRSA is an emerging public health issue; however, there is insufficient knowledge to guide effective treatments. NIAID is supporting 2 studies to determine the effectiveness of a variety of treatments for skin and soft-tissue infection. These studies will examine the duration of antimicrobial therapy using only oral, generic antibiotics and will determine the absolute need for antimicrobials and whether abscess drainage alone is a sufficient alternative. If successful alternative treatments are identified for CA-MRSA, then final-option drugs, such as vancomycin and linezolid, can be reserved for the treatment of highly resistant hospital-acquired MRSA infections. Through these clinical trials, NIAID facilitates the development of effective treatments for emerging infections.

Curbing antimicrobial resistance will require creatively addressing all facets of infectious disease prevention and treatment. NIAID will continue to support clinical trials that determine the optimal dosage and the value of substituting alternative treatments.

Working in partnership. In view of the inherent complexity of antimicrobial resistance and the need for research and clinical trials that range from bench to behavior, it is essential that multiple partners work together to address each aspect of the problem (figure 3). Success against antimicrobial resistance will require a multifaceted approach that includes increased surveillance, more judicious use of antimicrobials in both human medicine and agriculture, and increased research on the biology of the microbes, mechanisms of resistance, host response, vaccines, diagnostics, and therapeutics. NIAID has been engaged in several partnership efforts to further basic and applied research and support public health efforts to manage antimicrobial resistance, including the following:

- NIAID cochairs the federal government’s Interagency Task Force on Antimicrobial Resistance [27]. This task force is implementing an action plan to address the consequences of antimicrobial resistance.

\[\text{Figure 3.} \text{ Partnerships addressing antimicrobial resistance. The National Institute of Allergy and Infectious Diseases partners with numerous other agencies, institutes, and companies to coordinate efforts in combating antimicrobial resistance. WHO, World Health Organization.}\]
In 2007, NIAID played a key role in establishing the Not-for-Profit Partnership for TB, a public-private partnership between NIAID and the Singapore Economic Development Board to advance drug discovery for dengue fever.

NIAID contracted with the National Institute of Public Health and the Environment, the Centers for Disease Control and Prevention, the FDA, the Agency for Healthcare Research and Quality, the Department of Agriculture, the Department of Defense, the Department of Veterans Affairs, the Environmental Protection Agency, the Centers for Medicare and Medicaid Services, and the Health Resources and Services Administration.

In 2007, NIAID launched a new initiative, Partnerships with Public-Private Partnerships, to establish collaborations that will accelerate preclinical research and the development of products targeting neglected infectious diseases of global importance. Under this program, NIAID is partnering with the Medicines for Malaria Venture to develop a malarial drug that targets an essential enzyme of the malarial parasite, with the St. Jude Children’s Research Hospital to support a newly established antimalarial drug discovery consortium, with the Drugs for Neglected Diseases Initiative to establish a new international public-private partnership, the Lilly Partnership for TB, to emerge within the pharmaceutical industry with academic expertise in chemistry and TB microbiology, with the ultimate goal of developing new therapeutics effective against TB and MDR/XDR-TB.

In 2007, NIAID established a collaboration with the Novartis Institute for Tropical Diseases in Singapore (a public-private partnership between Novartis and the Singapore Economic Development Board) to advance drug discovery for dengue fever.

NIAID is addressing the problem of antimicrobial resistance by offering tools and resources to the scientific community to facilitate the highest-quality research and provide a flexible infrastructure to respond to emerging needs; supporting basic and translational research likely to lead to clinical applications that will reduce the prevalence of antimicrobial resistance; partnering with industry, other federal agencies, academia, and non-governmental organizations to take a comprehensive approach to the problem of antimicrobial resistance; encouraging development of broad-based vaccines and therapeutics that are effective against >1 pathogen; and supporting the development of multiplex diagnostics that will enable clinicians to make informed treatment choices.

Antimicrobial resistance is a perpetual challenge in our attempts to maintain a favorable balance between microbes and the human species. The efforts of NIAID and our partners from the public health, research, and medical communities are critical to addressing this challenge and thus maintaining this balance.

References