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

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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.

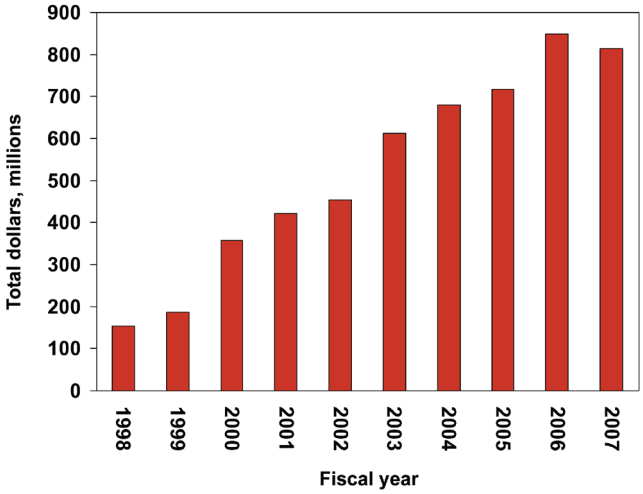

Figure 1. National Institute of Allergy and Infectious Diseases research funds for antimicrobial research. Bars reflect total dollars awarded in grants, cooperative agreements, contracts, and intramural research on antimicrobial research. This includes, but is not limited to, studies of mechanisms of 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.

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.

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

NIH-funded basic and preclinical research	Products in development	Stage of development
Efflux pumps	Efflux pump inhibitors that restore potency of antibiotics against drug-resistant gram-negative organisms	Preclinical
Efflux pump and ribosome protection	Broad-spectrum tetracycline-class antibiotics	Phase 2 clinical study under way for both oral and intravenous formulations in patients with complicated skin and skin structure infections
	Broad-spectrum macrolide antibiotic	Phase 2 clinical trial for treatment of community-acquired pneumonia
Ribosome targeting	Ribosome inhibitor	Phase 1 trials of first candidate initiated in December 2005
MAR regulon	Small molecule inhibitors of the MAR proteins that confer resistance	Proof of concept in *Escherichia coli, Yersinia,* and *Pseudomonas* by use of clinically relevant models of infection
Inhibitors of fatty acid synthesis	Novel antibacterial drugs	Lead identification/optimization stage
	TB drug that inhibits biosynthesis of the waxy outer coating of the tubercle bacillus	Preclinical studies (2005); entering phase 1 clinical trials
Alternative respiratory chain pathway	TB drug (nitroimidazole) that inhibits protein and cell wall lipid synthesis	Phase 2 clinical trials
Inhibitor of cell wall synthesis	TB drug that inhibits cell wall synthesis	Phase 1 clinical trials

NOTE. MAR, multiple adaptational response; TB, tuberculosis.

In recent years, there has been a growing interest in biofilms, which are structured communities of microorganisms enclosed in a self-produced hydrated polymeric matrix attached to a living or inert surface [13]. Although many infections manifest themselves as biofilms, most licensed antibiotics are not effective against them because of their sessile nature. NIAID is funding efforts to study the structure and physiology of biofilms in order to learn how to prevent or disrupt their formation. For example, 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 in the body 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*

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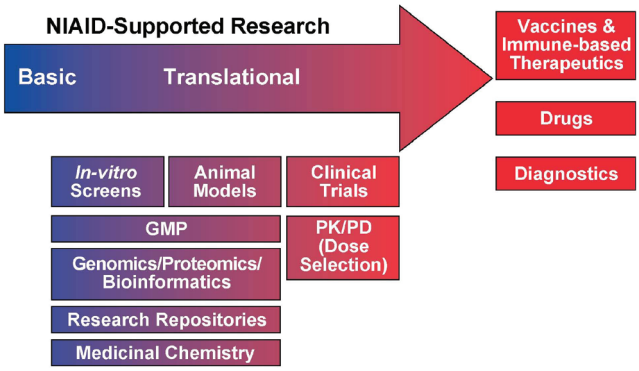

Figure 2. National Institute of Allergy and Infectious Diseases (NIAID) research resources for addressing antimicrobial resistance. The process of developing therapeutically useful products encompasses a range of activities from basic research *(blue),* through translational research, to the production 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.

gonorrhoeae, Pseudomonas aeruginosa, Salmonella typhimurium, S. aureus, Streptococcus pneumoniae, and *Streptococcus pyogenes,* as well as the fungal pathogen *Aspergillus fumigatus.* These resources not only serve the basic sciences but also facilitate the translation of basic findings into products that have clinical applications.

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 diagnosis, 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

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Table 2. Representative clinical trials that evaluate primarily off-patent drugs with strategies relevant to antimicrobial resistance.

Target infection	Antimicrobial agents	Objectives
Uncomplicated skin and soft-tissue infections caused by CA-MRSA	Clindamycin, trimethoprim-sulfamethoxazole, cephalexin, placebo	Preserve antimicrobials; assess need for antimicrobials; shorten course of antimicrobial therapy
Early stage syphilis	Azithromycin vs. benzathine penicillin	Evaluate single-dose therapy
Acute otitis media	Amoxicillin clavulanate vs. placebo	Assess need for antimicrobial therapy
Latent tubercular infection	Rifampin vs. isoniazid	Shorten course of antimicrobial therapy
Nongonococcal urethritis	Doxycycline, azithromycin, tinidazole	Evaluate combination therapy

NOTE. CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus.*

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 antimi-

Figure 3. Partnerships addressing antimicrobial resistance. The National Institute of Allergy and Infectious Diseases partners with numerous others agencies, institutes, and companies to coordinate efforts in combating antimicrobial resistance. WHO, World Health Organization.

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crobial resistance, including rising health care costs and increasing morbidity and mortality from certain infections. The task force is made up of representatives from NIAID, 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 develop a new drug to treat visceral leishmaniasis, and with the Trypanosomatid Drug Development Consortium to generate a robust pipeline of drugs to target trypanosome diseases.
- In 2007, NIAID played a key role in establishing a new international public-private partnership, the Lilly Not-for-Profit Partnership for TB Early Phase Drug Discovery. The aim is to integrate the medicinal chemistry expertise provided by 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 contracted with the National Research Council, part of the National Academy of Sciences, to conduct 2 workshops on infectious disease therapeutics. One focused on potential new classes of antibiotics; the other explored the possibility of treating infectious diseases by modulating the immune system. Workshop participants assessed the current state of knowledge, identified approaches in the development of antimicrobial therapeutics that have been successful in the past, and discussed ways in which new areas of research could revolutionize the treatment of infectious diseases [28].

CONCLUSION

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 nongovernmental 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;
- 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

1. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA **2007**; 298:1763–71.
2. Cardo D, Horan T, Andrus M, et al. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control **2004**; 32:470–85.
3. Jambou R, Legrand E, Niang M, et al. Resistance of *Plasmodium falciparum* field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. Lancet **2005**; 366:1960–3.
4. Zignol M, Hosseini MS, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. J Infect Dis **2006**; 194:479–85.
5. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs: worldwide, 2000–2004. MMWR Morb Mortal Wkly Rep **2006**; 55:301–5.
6. Shah NS, Wright A, Bai GH, et al. Worldwide emergence of extensively drug-resistant tuberculosis. Emerg Infect Dis **2007**; 13:380–7.
7. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet **2006**; 368:1575–80.
8. Moll A. Treatment of HIV associated XDR TB patients [abstract]. In: Program or abstracts of the 38th Lung Health Conference (Cape Town). **2007**.
9. D'Costa VM, McGrann KM, Hughes DW, Wright GD. Sampling the antibiotic resistome. Science **2006**; 311:374–7.
10. Spellberg B, Powers JH, Brass EP, Miller LG, Edwards J, John E. Trends in antimicrobial drug development: implications for the future. Clin Infect Dis **2004**; 38:1279–86.
11. Beg QK, Vazquez A, Ernst J, et al. Intracellular crowding defines the mode and sequence of substrate uptake by *Escherichia coli* and constrains its metabolic activity. Proc Natl Acad Sci USA **2007**; 104:12663–8.
12. Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. J Appl Microbiol **2002**; 92(Suppl):65S–71S.
13. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science **1999**; 284:1318–22.
14. Banin E, Brady KM, Greenberg EP. Chelator-induced dispersal and killing of *Pseudomonas aeruginosa* cells in a biofilm. Appl Environ Microbiol **2006**; 72:2064–9.

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15. Tang HM, Hamblin MR, Yow CM. A comparative in vitro photoinactivation study of clinical isolates of multidrug-resistant pathogens. J Infect Chemother **2007**; 13:87–91.
16. Foley JW, Song X, Demidova TN, Jalil F, Hamblin MR. Synthesis and properties of benzo[a] phenoxazinium chalcogen analogues as novel broad-spectrum antimicrobial photosensitizers. J Med Chem **2006**; 49:5291–9.
17. Voyich JM, Otto M, Mathema B, et al. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? J Infect Dis **2006**; 194:1761–70.
18. Labandeira-Rey M, Couzon F, Boisset S, et al. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. Science **2007**; 315:1130–3.
19. Wang R, Braughton KR, Kretschmer D, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med **2007**; 13:1510–4.
20. Network on Antimicrobial Resistance in *Staphylococcus aureus*. Available at: http://www.narsa.net/. Accessed 5 December 2007.
21. NIAID Microbial Sequencing Centers. Available at: http://www.niaid.nih.gov/dmid/genomes/mscs/. Accessed 25 February 2008.
22. Sarkar S, DeSantis ER, Kuper J. Resurgence of colistin use. Am J Health Syst Pharm **2007**; 64:2462–6.
23. United States Department of Health and Human Services. The Jordan report: accelerated development of vaccines 2007. Publication 06-6057. Bethesda, MD: National Institutes of Health, **2007**:157.
24. Schaffer AC, Solinga RM, Cocchiaro J, et al. Immunization with *Staphylococcus aureus* clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infect Immun **2006**; 74:2145–53.
25. Stranger-Jones YK, Bae T, Schneewind O. Vaccine assembly from surface proteins of *Staphylococcus aureus*. Proc Natl Acad Sci USA **2006**; 103:16942–7.
26. McKenney D, Pouliot KL, Wang Y, et al. Broadly protective vaccine for *Staphylococcus aureus* based on an in vivo-expressed antigen. Science **1999**; 284:1523–7.
27. Interagency Task Force on Antimicrobial Resistance. Available at: http://www.cdc.gov/drugresistance/actionplan/index.htm. Accessed 5 December 2007.
28. Treating infectious diseases in the microbial world: report of two workshops on novel antimicrobial therapeutics. Washington, DC: National Academies Press, **2006**. Available at: http://www.nap.edu/catalog/11471.html. Accessed 5 December 2007.

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