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Microbial resistance to growth inhibitors has been observed since the earliest days of chemotherapy, e.g., C.H. Browning’s work on trypanosomes in Paul Ehrlich’s laboratory. The biological basis of “drug-fastness” was controversial for many years. Some believed that the drug induced adaptive changes in the (then still obscure) genetic structures of the treated microbes; others proposed the random occurrence of resistant mutants which were merely selected by the drug. A variety of experiments in the decade 1943 to 1952, ranging from the fluctuation test introduced by Luria and Delbruck (1943) to indirect selection using replica plating (Lederbergs, 1952), provided abundant evidence for the selectionist theory. It was no surprise to the genetically informed that resistance became a growing problem not long after the introduction of sulfonamides and then penicillin into clinical practice.

Random mutation was by no means the whole story. Landmark studies by Tsutomu Watanabe and coworkers in the late 1950s uncovered resistance transfer factors (RTFs) in Shigella spp. that conferred resistance to multiple antibiotics, including streptomycin, chloramphenicol, tetracycline, and sulfonamide. In clinical practice, the borrowing of RTFs from other pre-existing resistant strains was often more important than the selection of de novo mutants in the treated host, especially for multiple drug resistance. The RTFs fit into an overall framework of infective heredity, founded on the prevalence of plasmids (extranuclear autonomous fragments of DNA that allow for lateral transfer of genetic information across strains, species, and even phyla and that may encode any part of the genome, including tumorigenesis and conjugational competence genes).

The discovery of RTFs gave Dr. Stuart B. Levy the impetus to work in Watanabe’s laboratory and to embark on a career devoted to the study of antibiotic resistance and its public health impact. While this public health problem was originally confined to Japan, it was realized early on that it would be only a matter of time before it became a worldwide threat. Stuart’s contributions to the field of antibiotic resistance over the past four decades have included many important discoveries regarding various resistance mechanisms. His pioneering efforts in the arena of tetracycline resistance led to the seminal discovery of active drug efflux as a mechanism of resistance. Stuart is also credited with the discovery of the intrinsic (chromosomal) and inducible multiple antibiotic resistance (mar) locus in Escherichia coli. This system regulates susceptibility to multiple antibiotics, oxidative stress compounds, detergents, organic solvents, and biocides through a network of genes termed the Mar regulon (but primarily involving mechanisms of decreased drug uptake and increased drug efflux). These findings have helped to create new avenues in the field of intrinsic drug resistance and have led to important new understandings of the bacterium’s natural defense mechanisms to noxious stimuli. The parallels between bacterial tetracycline-specific efflux mechanisms and multiple antibiotic efflux mechanisms led Stuart to investigate whether similar mechanisms were operative in eukaryotic cells during growth in the presence of cancer chemotherapeutics.

Early studies on selective antimicrobial pressure in the environment, performed in the Levy laboratory at Tufts University School of Medicine, are considered groundbreaking experiments on the subject. They have fostered a large body of ongoing research on agricultural antimicrobial use, which continues to be pursued today. Over 30 years ago, Stuart led a prototypic prospective study on the effects of antibiotic-laced feed on the carriage of antibiotic-resistant bacteria in farm animals and staff that was published in the New England Journal of Medicine. Subtherapeutic amounts of oxytetracycline, often used as a growth promoter for chickens, led to their colonization by
Within 3 to 5 months, farm staff were excreting high numbers of tetracycline- and multidrug-resistant *E. coli* as well. Thus, feed for chickens impacts the flora carried by neighboring humans and, surely, the overall environment. One recent estimate put the tonnage of antibiotics used in animals and animal feed in the millions (M. Mellon, C. Benbrook, and K. L. Benbrook, *Hogging It: Estimates of Antimicrobial Abuse in Livestock*, UCS Publications, Cambridge, United Kingdom, 2001), matching the amount used for human therapeutics (S. A. McEwen and P. J. Fedorka-Cray, *Clin. Infect. Dis.* 34:S93-S106, 2002).

Stuart is recognized as one of the leading researchers in the tetracycline class of agents. He was among the first to show genetic heterogeneity among drug resistance determinants by defining different tetracycline resistance genes. Moreover, in some of the earliest studies in molecular epidemiology, he showed the distribution of different tetracycline resistance determinants among bacteria. His team led the effort to produce a nomenclature system for the different tetracycline resistance determinants, which is regularly revised with new discoveries. Stuart's more recent laboratory forays have led him into studies designed to evaluate resistance to household disinfectants and biocides. Pine oil, a common component of detergents and other household cleaning fluids, was used initially by Stuart and colleagues as a selective agent to recover resistance to multiple antibiotics in vitro. Biocides, including agents such as triclosan, were previously thought to kill bacteria by physical disruption. Well-crafted laboratory experiments convincingly demonstrated that triclosan does indeed have a specific site of action within the cell at a protein (FabI) that is involved in fatty acid biosynthesis. These studies imply that the overuse of antibacterial-containing household products could lead to the unintended selection of antibiotic-resistant bacteria.

The optimism of the early period of antimicrobial discovery has been tempered by the emergence of bacterial strains displaying resistance to multiple antimicrobial agents, an unfortunate legacy of past decades of antimicrobial use and abuse. As a practicing physician and scientist, Stuart has been an ardent spokesperson for the recognition of this problem over the past 30 years. Drug resistance presents an ever-increasing global public health threat that involves all major microbial pathogens and antimicrobial drugs. The scarcity of new antimicrobials on the horizon and the increasing prevalence of multidrug resistance mean that we must redouble our efforts to judiciously preserve the agents at hand, while intensifying the search for new therapeutics. For many years, Stuart was a lone voice speaking out for prudent use of antibiotics while receiving little responsive action. In the present day, he continues to serve as a passionate advocate for alternatives to growth-promoting antimicrobials in food animal production, as well as for prudent antibiotic use in all settings. The public health consequences of a misguided inclination for “sterilizing” our environment with biocides and other broad-spectrum household antimicrobials, in terms of potential adverse effects on microbial ecology and drug resistance, have become a contemporary point of concern for Stuart. He speaks and writes on the need for greater awareness of how our short-sighted overuse and sometimes misuse of these therapeutics generate multidrug-resistant bacteria that today confront clinicians and patients worldwide.

His message, directed initially to professional groups, has moved to the public in the form of lectures and his widely cited book *The Antibiotic Paradox: How Miracle Drugs Are Destroying the Miracle*. First published in 1992, this book has since been updated and translated into French, Korean, and Chinese. Stuart’s dedicated leadership in the policy and education arenas is also exemplified by the Alliance for the Prudent Use of Antimicrobials (APUA) which he founded in 1981. APUA is a growing international organization with a presence in 100 countries and networks of chapters and individuals working closely on communication and research to foster prudent antibiotic use and to curtail antibiotic resistance. They recently published a report entitled “Shadow Epidemic,” which quite clearly illustrates the high prevalence of resistance to front-line agents in common microbial pathogens. Stuart’s message reaches wide audiences through print and visual media and appearances before national and international audiences. Now through this publication, Stuart’s colleagues, collaborators, and students have the opportunity to honor his past and present achievements and, perhaps of greatest import to him, to carry his message still further.

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Antimicrobial resistance in pathogenic microorganisms is recognized as one of the chief threats to human health worldwide. While antimicrobial resistance has long been recognized as a biological phenomenon, early observations were generally considered interesting laboratory events with little, if any, clinical relevance. Even when resistance became more common, the advent of new anti-infective agents was able to outpace incipient resistance phenotypes, which typically appeared at low frequency and increased in usually small increments. Today, the situation is much different. Beginning in the late 1980s, a large number of resistance phenotypes began to appear, and there is now widespread resistance to many antimicrobial agents, with multiple antimicrobial resistance phenotypes being the rule rather than the exception for many pathogens. More recently, multidrug resistant pathogens indigenous to the hospital environment have moved into the community (e.g., methicillin-resistant *Staphylococcus aureus*). The CDC estimates that 70% of bacterial infections are caused by organisms resistant to at least one antimicrobial, and the Institute of Medicine estimates the total annual cost of antimicrobial resistance in the U.S. at nearly $5 billion.

Some researchers recognized the signs and the potential pitfalls of indiscriminate antibiotic use early on and persisted for years in explaining the looming problem to a largely silent audience. One such pioneer was Dr. Stuart B. Levy. In addition to providing a thorough review of key pathogens and drug resistance in various classes of antimicrobials, this book is also intended as a tribute to Stuart’s unique contributions to the field. Through his own diligence, imagination, and charisma, he has combined basic microbiology research with public education in an ongoing effort to limit antimicrobial resistance, to preserve the power of these precious natural resources, and to offer new therapies to future generations. The enthusiastic replies we received from the contributors when first proposing this project are a testimony to Stuart’s influence on the science of resistance and the scientific community.

The sections in this book were chosen with the goal of providing a comprehensive overview of antibacterial and anticancer drug resistance, with special emphasis on those areas where Stuart stimulated scientific advancements through his own research and that of his students, research fellows, and colleagues. Thus, the authors of the respective chapters have either spent time in his laboratory or collaborated with him on research, policy, or educational initiatives.

This book is divided into 7 sections and 40 chapters that focus on the current knowledge of mechanisms of antimicrobial and anticancer drug resistance, the ecology of resistant bacteria, the factors influencing antimicrobial resistance, and efforts in developing and implementing relevant policy and education. While the book is unified in theme, each chapter is meant to stand alone as the author’s own perspectives on the resistance problem. We are confident that the reader will find these chapters to be an excellent review of the subject.

In addition to the contributing authors and a dedicated ASM publication staff, the editors would like to thank Neil Woodford, Marcus Zervos, Christopher Ohl, Barry Hurlburt, Judah L. Rosner, David Hecht, Tom Shryock, Douglas Hutchinson, Kenneth Thompson, Qijing Zhang, and Steven Projan for critical review of portions of this volume.

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April 2005
I am honored and somewhat awed by this book. To see so many of my colleagues, associates, and students contributing to this compendium of knowledge and insight into the drug resistance field is more than any research scientist can ever expect. What an impressive integration of findings, concepts, and new ideas provided by so many distinguished investigators in this broad and multifaceted field!

My decision to pursue a research career in microbiology was largely influenced by my introduction to the phenomenon of antibiotic resistance in 1963. I was spending a year away from medical school as a predoctoral student with Professor Raymond Latarjet at the Laboratoire Pasteur of the Institut du Radium in Paris. In a journal club, I was fascinated to learn about R factors, curious means of drug resistance transfer among bacteria of different genera. I decided to understand firsthand the findings in this field. I wrote to Tsutomu Watanabe, an early pioneer in drug resistance research to ask if I could come work with him during the summer of my third year of medical school. I was delighted to be accepted and to spend several months in 1964 in his laboratory at Keio University in Tokyo, Japan.

Side-by-side with him at the bench or singing with him and his group after work at a karaoke restaurant, we established a strong friendship and collegiality up until his untimely death in 1973. He remains an influence on me. I am passionately taken by the mysteries of drug resistance and am not alone, as evidenced by this book. The problem has engaged individuals in multiple disciplines, including genetics, molecular biology, biochemistry, pharmacology, epidemiology, ecology, and evolution. There is also, of course, the economic cost as well as the public and private health impact of resistance in the treatment of infectious diseases. More and more, we understand the globality of resistance—how easily resistance genes can move among genera and species and how resistant bacteria spread from country to country.

Another individual, Joshua Lederberg, also influenced my entry into this field. His discovery of gene transfer among bacteria formed the foundation for understanding the transfer of drug resistance on R factors. I had a chance meeting with him in Stanford, Calif., when I was looking for residency programs. Josh has remained a friend, a valued supporter of prudent antibiotic use, and a steadfast active participant in the infectious disease area. Among other early pioneers with whom I have had the pleasure to interact are Richard Novick, Julian Davies, and Stanley Falkow. Their studies and outreach activities stimulated and provided a firm foundation for many of us in the field.

I thank Michael Alekshun, Patrick McDermott, and David White for their organizing and steerage of this book and their continued dedication to the drug resistance problem. Each of these researchers has achieved recognition for independent accomplishments in the field. I am proud and pleased to have hosted them in my laboratory. For many years, I have argued for more attention to drug resistance, not just from the vantage point of public health, but also from my concern that the antibiotic resistance field was losing investigators and failing to attract new ones, even as the problem magnified. Mike, Pat, and David are fine examples of the next generation.

On a larger scale, it is gratifying to see that a new Study Section on Drug Discovery and Mechanisms of Antimicrobial Resistance has been formed at the National Institutes of Health. This action alone in response to urging by many of us will encourage new investigators to enter the field and support those already in it.

I am also indebted to my long-standing colleague Laura McMurry, who for three decades has...
worked closely with me in our studies, particularly on the Tet protein and the mar regulatory locus. My second “right hand” is Bonnie Marshall, whose expertise in clinical microbiology has been complemented by her striking ability to skillfully learn genetics and molecular biology. She has been there for whatever ecology study we have undertaken, whether it be spread of bacteria and plasmids on a farm, release of bacteria from autoclaves, biocide impact on household microbiology, or contamination of wind instruments. Finally, my third long-standing valuable support is Mark Nelson, whose willingness to work, often alone, in identifying and creating new tetracyclines provided the means to move forward in the tetracycline “renaissance.” This effort eventually led to the founding of Paratek Pharmaceuticals, among whose aims are to identify new tetracyclines not subject to current resistance mechanisms and to uncover novel approaches to prevent and treat infectious diseases.

Our knowledge of antibiotic resistance has come a long way. The genetics of resistance and its causative transfer elements were just being discovered in the 1960s when I was first introduced to the problem. Over these past four decades, we have learned about new genetic elements, new transfer mechanisms, new kinds of resistances, new organisms, and new insights into the origins of some resistance genes, particularly among soil organisms. More importantly, we have reached a period when antimicrobial resistance is finally receiving the close attention that it merits from government and non-government organizations and individuals. The problem has climbed to the priority list of most public health agendas. Several of us place it at the pinnacle, since it impacts so many areas of infectious diseases and health. As the Alliance for the Prudent Use of Antibiotics states, drug resistance is a disease in itself—a “shadow epidemic”—which casts a disheartening gloom over the successful treatment of all kinds of infectious diseases throughout the world.

Nonetheless, I believe we are at the beginning of an exciting new era, hopefully replete with new molecular findings in drug resistance, new drugs for the physicians’ armamentarium, and a more expansive appreciation of the power of microbes. We need to approach infectious diseases with a new paradigm that seeks control, not elimination, of microbes and disease prevention, not sterilization of our environment. The contributors to this volume are among the leaders facing these issues and providing the knowledge needed to find a solution to this important health challenge.

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Jay and Stuart Levy, Centennial ASM Celebration, Chicago, Ill., 1999


The Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 2002. Row 1: Carol Kumamoto, Cathy Squires, Joan Mecsas; row 2: Dean Dawson, Stuart Levy, Linc Sonenshein, Claire Moore; row 3: Edward Goldberg, Ralph Isberg, Andrew Camilli, John Coffin; row 4: Matt Waldor, Mike Malamy, David Lazinski, Ted Park, Andrew Wright.

Still together . . . Laura McMurray (since 1971), Stuart, and Bonnie Marshall (since 1977)
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