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Solving the Antibiotic Crisis

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ABSTRACT: Antibiotics are essential for both treating and preventing infectious diseases. Paradoxically, despite their importance as pillars of modern medicine, we are in danger of losing antibiotics because of the evolution and dissemination of resistance mechanisms throughout all pathogenic microbes. This fact, coupled with an inability to bring new drugs to market at a pace that matches resistance, has resulted in a crisis of global proportion. Solving this crisis requires the actions of many stakeholders, but chemists, chemical biologists, and microbiologists must drive the scientific innovation that is required to maintain our antibiotic arsenal. This innovation requires (1) a deep understanding of the evolution and reservoirs of resistance; (2) full knowledge of the molecular mechanisms of antibiotic action and resistance; (3) the discovery of chemical and genetic probes of antibiotic action and resistance; (4) the integration of systems biology into antibiotic discovery; and (5) the discovery of new antimicrobial chemical matter. Addressing these pressing scientific gaps will ensure that we can meet the antibiotic crisis with creativity and purpose.

KEYWORDS: *antibiotic resistance, evolution, reservoir, natural products, systems biology, drug discovery, mechanism*

Resistance to antibiotics and antimicrobial agents is now a major concern for public health agencies and leaders across the globe. The World Health Organization,[1] the U.S. Centers for Disease Control and Prevention,[2] the White House,[3] and government organizations in the United Kingdom[4] and Canada[5] have all released recent reports describing the crisis in antibiotic resistance and the scarcity of new antibiotic drugs in the face of this growing clinical need. In Canada, infections caused only 3% of deaths in 2009, whereas in 1925 the number was 56%;[6] antibiotics are a large reason for this revolution in health and for the longevity we now enjoy. The ability to control infection through the use of antibiotics is also the cornerstone of a great number of the interventions in modern medicine that we have come to take for granted. It is impossible to imagine cancer chemotherapy or organ transplantation that both result in suppression of the immune system without antibiotics, just as it is impossible to expect infection-free major surgeries without these drugs. Yet without new antibiotics to counter increasing rates of resistance, we will soon be in a postantibiotic era that will change medicine profoundly.

There are three major challenges to bringing new antibiotic drugs to market. These include first the economics of modern drug discovery that discourages investment in antibiotics over other drug classes; second, challenges in the demands of the clinical trial system required for approval of new antibiotics, especially those directed toward resistant pathogens;[7] and, third, significant scientific difficulties in identifying candidate new drugs, their targets, and innovative new solutions to the antibiotic crisis.

There have been proposals to address the first two challenges, and there are a growing number legislative efforts such as the Generating Antibiotics Incentives Now (GAIN) Act passed into law in the United States in 2012 to intervene in the hopes of providing the right conditions to stimulate investment in antibiotic discovery.[8] Most chemists and chemical biologists have little aptitude or influence in these political and regulatory spheres, but where we can, and must, make a transformative difference is in addressing the third challenge of scientific innovation. The issues of economics and regulation will eventually be solved, but unless there are lead molecules and new therapeutic strategies poised to take advantage, we will lose the battle against resistance.

The time is right for a call to arms in the scientific community to tackle fundamental and applied aspects of antimicrobial research to ensure that we can address the significant scientific gaps that are responsible for our lack of progress in delivering new antibiotic leads into clinical development. In particular, we need research and development in five key areas (Figure 1):

1) deep understanding of the evolution and reservoirs of resistance,
2) full knowledge of the molecular mechanisms of antibiotic action and resistance,
3) discovery of chemical and genetic probes of antibiotic action and resistance,

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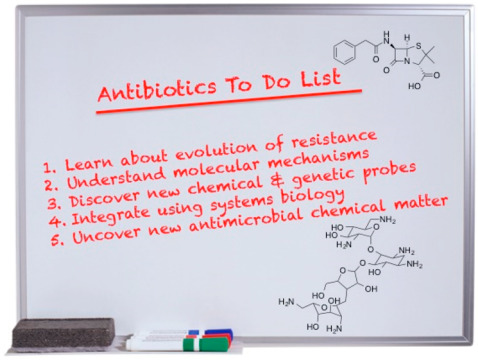

Figure 1. An antibiotics "to do" list. Research and development in five key areas are vital to help solve the antibiotics crisis.

4) integration of systems biology into antibiotic discovery, and
5) discovery of new antimicrobial chemical matter.

1. Evolution and Reservoirs of Antibiotic Resistance. For the most part, pathogens are sensitive to antibiotics. Many "pathogens" are normal or transient members of the human microbiome and cause disease only after acquiring genes that impart virulence characteristics and/or they move opportunistically to organs that they do not normally colonize, for example, when the skin or intestinal tract is perforated.[9] In contrast, environmental bacteria tend to be highly antibiotic resistant.[10] This reflects the different settings bacteria have evolved in. Bacteria of the human microbiome simply do not normally interact with the massive chemical diversity of soil or aquatic environments. As a result, their intrinsic antibiotic resistomes[11] are not adapted to detoxify as broad a spectrum of chemical diversity as microbes from the environment. We now know that the majority of resistance elements in pathogens acquired by horizontal gene transfer have their origins in environmental bacteria.[12] The extent of this environmental resistome is unknown, as are the factors that stimulate gene transfer. We do know that certain environments (often of human origin such as wastewater treatment plants) are "hot spots" for gene transfer,[13] but the molecular mechanisms of gene movement are largely enigmatic. We need a full accounting of the environmental antibiotic resistome (the collection of all genes linked to resistance) as well as the roles these genes and their products play in the environment along with a thorough understanding of gene transfer among microbes. This fundamental knowledge is essential for our understanding of how resistance evolves as well as the selection pressures that lead to the distribution of associated genes among organisms.[10c] This information will provide an early-warning system for the emergence of resistance in the clinic and inform efforts in antibiotic stewardship.

2. Mechanisms of Antibiotic Action and Resistance. Antibiotics continue to surprise us. We have historically classified them according to chemical class and mode of action, for example, β-lactams that target cell wall synthesis or tetracyclines that block translation. Over the past decade, with breakthroughs in determining the details of the structure and function of many antibiotic targets such as the ribosome, we have an unprecedented view of the atomic details of antibiotic action. However, there is still much work to be done. The molecular targets are known for most of our clinically used drugs, but the precise mechanisms of cell death are often mysterious. Although we have known for decades that β-lactam antibiotics target cell wall biosynthetic penicillin-binding proteins (PBPs), recent work demonstrates that inhibition of these enzymes results in downstream effects on cell wall synthesis and degradation that drain metabolic resources contributing to cell death.[14] This opens up new possibilities for enhancing antibiotic activity and possibly new targets to exploit. The research (and controversy) on the role of reactive oxygen species in antibiotic-mediate cell killing is another example of filling in the knowledge gaps of how antibiotics actually work.[15] For the thousands of antibiotics that have been discovered over the years, but not clinically implemented, we know very little about their activities. Surely there this is a rich vein here of target space to mine for next-generation antibiotic leads. The objectives should be to link the molecular targets and mode of action of every antibiotic class with exemplars of the associated chemical species.

Reciprocally, detailed mechanisms of resistance are poorly understood. In particular, the efflux systems that dominate in Gram-negative bacteria are only now offering up their molecular details thanks to breakthroughs in structural biology.[16] The regulation of these genes is also of paramount importance as is the hierarchy of expression in genera such as *Acenitobacter* and *Pseudomonas* that harbor multiple paralogues in their genomes. The intrinsic resistomes of pathogens that prevent us from deploying our existing drugs are comprised by many redundant pathways, which offer potential targets for antibiotic adjuvants.[17] Miller's pioneering efforts to systematically explore the *Escherichia coli* intrinsic resistome[18] deserve to be replicated in other organisms.

Furthermore, how resistance genes move through bacterial populations via mobile elements is also of great importance and a potential target for reducing the spread of resistance. The molecular mechanisms of this "mobilome"[19] go largely unexplored and are at the root of the challenge of acquired resistance in the clinic.

3. Chemical and Genetic Probes of Antibiotic Action and Resistance. Genomic and chemical tool sets are essential to expand our knowledge of the mechanisms of antibiotic action and resistance. Mori's Keio[20] and ASKA[21] collections offer complete in-frame single-gene deletion and gene expression sets for *E. coli*. Such powerful tools should be available for all key human pathogens. An effort to generate such gene sets for the remaining ESKAPE pathogens[22] (*Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acenitobacter*, *Pseudomonas*, *Enterobacter*), for example, would have a transformative effect on antibiotic research. Transposon insertion mutant libraries already exist for some of these[23] and have been used to explore intrinsic resistance,[24] demonstrating the value and power of such comprehensive genetic tools. Integrated databases of gene function and links to antibiotic action would also be transformative, bringing bioinformatic tools that other fields such as yeast molecular biology have used to great advantage on the antibiotics area. Efforts to build such databases for resistance[25] need to be linked to target information to provide comprehensive informatic coverage of antibiotic activity.

Similarly, small molecule inhibitors and probes of antibiotic mode of action and resistance will be of great value. Such compounds could even serve as lead scaffolds in antibiotic design and as starting points for antibiotic adjuvants — small molecules that enhance the activity of antibiotics.[26] For example, efforts to block the activity of resistance and rescue antibiotics in the clinic continue to be successful for the β-

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lactam antibiotics[27] but so far not for other antibiotic classes. A concerted effort to identify inhibitors of antibiotic resistance elements will generate a collection of chemical probes and leads essential to adjuvant discovery. Another approach to identify antibiotic adjuvants that can inform on antibiotic action is through screens of small molecule libraries that enhance activity followed by determination of their targets.[28] Such compounds can help to pinpoint the targets of antibiotics of unknown function[29] and can also expose the vulnerable intrinsic resistome of bacteria. We should strive to generate an arsenal of such compounds along with a detailed understanding of their mechanisms to inform drug discovery efforts and as chemical biological tools for deeper understanding of antibiotic action.

4. Integration of Systems Biology into Antibiotic Discovery. Traditional antibiotic drug discovery has relied on a cell death phenotype screen, where compounds were systematically arrayed versys panels of pathogenic bacteria to identify hit compounds for further development.[30] When that approach, which identified essentially our entire current antibiotic arsenal, failed to deliver new leads in the late 20th century, the field turned to target-based approaches where single essential protein targets were screened in vitro for inhibitors followed by lead optimization. Unfortunately, this approach has yet to identify new antibiotic drugs.[31]

In the meantime, our understanding of microbial systems biology has grown dramatically. In this work, largely emerging from the yeast research community, it has become apparent that the essential cellular processes that are considered the best targets for antibiotics are often highly buffered by redundant pathways that protect the cell from disruption of critical pathways. For example, in yeast, whereas only ∼20% of the genome is "essential", that is, genes that cannot be inactivated and still support life, there are orders of magnitude more synthetic lethal interactions, which are pairs of otherwise nonessential genes that cannot be inactivated.[32] Mapping these interactions offers a whole new target landscape for compounds that may block more that one cellular target[33] or pairs of compounds that in combination uncover cryptic synergy. In fact, the β-lactam antibiotics, arguably the most successful and essential antibiotics in clinical use, are examples of the former, where they inactivate many PBPs that together result in inhibition of cell wall synthesis. Inhibition of the intrinsic resistome is an illustration of the combination approach,[28,34] although this strategy is not limited to this example.

Approaching antimicrobial drug discovery with a systems biology mindset greatly expands the target vista. The strategy also enables new thinking about drug discovery toward a narrow spectrum of pathogens. Traditionally, antibiotic discovery programs select for lead compounds that are active against a broad spectrum of pathogens. Because the treatment of bacterial infection is very often empirical, where the prescriber does not know the nature of the actual infectious organism, broad-spectrum antibiotics make great sense. However, this approach provides the means for selection of resistance in many organisms and also has the potential for off-target effects that damage the microbiome, sometimes with devastating effect, for example, *Clostridium difficile*-associated colitis.[35] With improvements in rapid and accurate diagnostics, there is increasing understanding that narrow-spectrum or even species-specific antibiotics are achievable. Because the cellular networks of bacteria share many nodes and pathways but importantly differ in critical ways (one of the reasons they are in fact distinct species), this offers an opportunity to target networks that are specific to a given pathogen in antibiotic discovery. What we need is deep fundamental research in these networks along with small molecule probes to investigate them.

The systems biology approach also facilitates exploring alternative targets not associated with cell death. For example, genes required for virulence, formation of biofilms, enabling of antibiotic insensitive states such as persistence, etc., all can be investigated within a screening and informatics framework that is grounded in systems biology thinking.

5. Discovery of New Antimicrobial Chemical Matter. Antibiotics defy the paradigms of what chemical matter is appropriate for drug discovery. In particular, antibiotics do not follow such criteria as Lipinski's Rule of Five (as Lipinski himself pointed out).[36] This reflects the unique biology and intrinsic small molecule resistance of bacteria that is born of their evolution over millennia. In retrospect, it is therefore not surprising that screens of libraries of small molecules that were compiled with such criteria in mind have generally not yielded powerful antibiotic leads over the past two decades. Such compounds, though, may yet show activity as adjuvants or in combination screens, and it is important not to discount this region of chemical space going forward.

On the other hand, natural products, in particular those of microbial origin, have been the source of most of our antibiotics now in clinical use. Berdy estimated that between 1950 and 2002 20,000 bioactive microbial natural products were identified, yet only a very few of these (<20) chemical scaffolds were entered into clinical use as antibiotics.[37] The remaining compounds were not pursued for a number of reasons (toxicity, availability, specificity, solubility, etc.). No doubt, there are great opportunities to revisit these compounds now with the molecular tools and urgency of the clinical needs of the second decade of the 21st century. Unfortunately, most of these compounds are not readily available and the source organisms lost or unavailable. A systematic effort to create libraries of these bioactive molecules and make them available to the antibiotic research community would offer a treasure trove of probe molecules to inform on antibiotic action and even act as leads for new drugs.

The major drawback of looking back to microbial natural products in antibiotic discovery is the dereplication problem, that is, the rediscovery of known molecules. Using the cell death phenotype screen, all antibiotics look the same. Subsequent activity-guided purification from natural product extracts to identify the active antimicrobial compound, which is the most resource-consuming aspect of this procedure, very often identifies known molecules. Dereplicating these extracts to enrich in hits that yield novel chemical scaffolds is a technical challenge that is being met using a variety of techniques.[38] Additional innovation here would greatly improve the palatability of revisiting natural products in antibiotic discovery.

Another route to new chemical diversity is exploration of new genetic diversity. Most of the antibiotics we have in hand are derived from genera of microbes that grow readily under laboratory conditions. Firmicutes such as members of the genus *Bacillus* and Actinobacteria such as the *Streptomyces* are readily cultured from the soil and were the first sources of antibiotics, for example, gramicidin from *Bacillus brevis* identified by René Dubos in 1939[39] and streptomycin from *Streptomyces griseus* collected by Selman Waksman in 1944.[40] Bacteria from these phyla have been repeatedly explored since these pioneering efforts to great effect, generating most of our antibiotics, as well as many anticancer and immune-suppressing drugs. However,

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many individual species produce the same antibiotics or minor variations of the core scaffold; therefore, identifying new chemical scaffolds requires enormous screening efforts. The exploration of harder to cultivate genera, on the other hand, offers a potential entry point into new chemistry. For example, in recent work, Lewis's group reports the identification of a new inhibitor of lipid precursor metabolism that is required for cell wall synthesis with a novel chemical scaffold.[41] The compound, which shows no resistance thus far, was isolated from a new strain of betaproteobacteria using a technology that enriches for difficult to cultivate species by in situ growth in the natural environment of the microbe.

The existing 20000+ natural products already in the literature,[37] though, still provide a great starting place for expanding chemical diversity. Traditionally, this has been achieved using semisynthesis; however, synthetic biology platforms are increasingly improving with the commensurate ability to greatly enhance chemical diversity.[42] The genetic programs encoding microbial natural products, with only a few exceptions, are clustered together in the genome of the producing organism. This enables ready identification of the cluster and even prediction of the compound structure.[43] As algorithms for structure prediction improve, mining of genomic and metagenomic data for new scaffolds will become easier. Mobilizing these clusters into surrogate producers, technology that is presently in its infancy, will greatly facilitate access to novel chemistry and its manipulation. Such a synthetic biology approach offers nearly limitless ability to generate libraries of compounds that have the potential to greatly expand bioactive chemical space and also increase chances of identifying new antibiotics, antibiotics adjuvants, etc. These technologies need to be pursued with vigor.

CONCLUSIONS

The antibiotic crisis demands that we find creative solutions to avoid drifting back to a preantibiotic era where infectious diseases predominate as the cause of death and degradation of quality of life. Changing the economic and regulatory barriers to favor new antibiotic drug discovery requires force of will and imagination from leaders in the spheres of politics, government, and public health. These are achievable and becoming more likely as clinicians increasingly face the impact of the lack of new antibiotic drugs at their disposal in the face of ever-more resistant pathogens. The scientific obstacles, on the other hand, are the domain of researchers in fields such as chemistry, chemical biology, and microbiology. Only we can solve these problems, and as a community we need to marshal our best efforts to provide the knowledge, strategies, and compounds that can be harnessed to develop the next generation of antibiotics. The bad news is that we need to move quickly on many fronts to achieve these goals. The good news is that we are equipped, as never before, with powerful scientific tools as well as knowledge of history to address one of the 21st century's first serious tests of our commitment to innovation in health.

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Notes

The authors declare no competing financial interest.

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