

Antimicrobial Resistance — A New Drug Discovery Perspective Using Synthetic Biology**

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Summary

Antibiotic resistance is a significant international health issue that needs to be addressed and solved urgently. However, major pharmaceutical companies have reduced their efforts in novel antimicrobial discovery because of the low cost-to-profit ratio and the difficulty in actually finding new compounds with effective activity. Synthetic Biology (SB) will offer a new approach to the discovery of novel drugs, overcoming some of these problems. Using this state-of-art technology, antibiotics can be designed for novel activity and can be developed much more cost effectively. For SB to be fully exploited for discovering new antimicrobials, a number of policy changes are required (e.g., new funding mechanisms, more international collaboration, dedicated funding for high-risk fundamental research, stronger translation of new technologies to industry, new funding/research initiatives targeted at less-affluent country markets).

Current realities

The availability of antimicrobial drugs has dramatically changed the public health landscape since the 1940s. Serious diseases, which led to almost certain disability or death in a large number of patients, are now rapidly and safely cured with antibiotics. We now understand that microbial populations in the pre-antibiotic age already maintained a very low level of natural antibiotic resistance, and the treatment with antibiotics quickly selected the resistant strains, with rapid exchange of resistance genes leading to ever more intractable microbes. This mechanism is particularly effective and serious in hospital settings, and several generations of newly developed antimicrobials are already ineffective against a large fraction of hospital-acquired infections.

We are now threatened by the emergence of microbes that are resistant to all available antimicrobial drugs. This would potentially facilitate a return to the pre-antibiotic situation, where a simple scratch or minor cold could develop into a life-threatening disease that could kill large parts of the population, especially amongst the very young and elderly. Chronic misuse of antibiotics, for example in animal husbandry or due to over-the-counter availability, accelerates the emergence of resistant pathogens.

The obvious challenge for drug discovery is to rapidly and continuously come up with new generations of antimicrobial drugs that overcome the resistance mechanisms and provide a new line of defense. However, at the same time that antimicrobial resistance is on the rise, we see a dramatic decline in the number of new antibiotics entering the market (Figure 1B), and currently the pipeline of new agents reaching the market is almost empty, especially for the treatment of gram-negative infections by bacteria such as *E. coli*, *Salmonella*, *Pseudomonas* and *Shigella*, all of which cause significant morbidity and mortality. Drugs with completely new modes of action are rarely becoming available, with new developments usually being based on derivatives of existing antibiotics. Only two new antibiotics entered the market in the last decade: telavancin, which was approved by the Food and Drug Administration (FDA) in September 2009, is a vancomycin derivative; and ceftaroline, approved in October 2010, which is a cephalosporin with a mode of action similar to penicillin; both are used to treat multi-drug resistant skin infections.

There are two main reasons for this. First, it is economically no longer attractive to develop new antibiotics: these are drugs that (if they work) are taken for a few weeks only, and then the patient is cured. Even if drugs for the treatment of multi-drug-resistant hospital-acquired infections can be sold at a premium price, they pose difficulties earning back the immense costs associated with taking a new drug through the development pipeline (clinical tests, regulatory procedures, marketing).

Second, traditional methods of searching for new antimicrobials have lost their effectiveness. Large-scale screening procedures tend to re-discover the same candidates repeatedly, further increasing the economic risk involved in the search for new antimicrobials.

Scientific opportunities and challenges

SB is defined as the use of new genome synthesis technologies to create new living systems with beneficial functionalities. This is a new approach to antibiotic discovery, which has the potential for discovering truly novel antimicrobials at the rate required by the rapid emergence of resistance after the introduction of each new drug. It exploits the fact that the vast majority of antibiotics are actually produced by microbes, using genetically encoded modular enzymatic “assembly lines,” which are particularly amenable to an engineering approach to biology. SB has the additional benefit of being a technology that can be used in academic research, moving the main drug discovery work from large pharmaceutical companies toward academia (and small biotech companies).

Genome sequencing of thousands of bacteria and fungi has revealed that each species of microbe tends to contain the machinery to produce antibiotic compounds. Some soil bacteria are predicted to be able to produce several dozens of different antimicrobials. However, it has also become clear that the largest fraction of these potentially new compounds is invisible in classical screening: the biosynthetic machinery is “asleep” or silent (i.e., their potentially novel end-products are not detectable under the usual culture conditions in which microbes can be grown). The challenge for SB is to create new producer microbes that would “awaken” the silent biosynthesis of new antimicrobials and produce them in large and robust amounts. The general strategy has the following steps (Figure 1B):

1. Identify the biosynthetic machinery for new antimicrobials by large-scale genome sequencing of a large number of diverse organisms from distinct niches.
2. Isolate the responsible genes and put them under artificial, engineered control systems, so they can be activated as needed.
3. Transfer the engineered gene cluster into a host bacterium, which has been specifically designed to provide the necessary components for antibiotic biosynthesis in large amounts.
4. Isolate, identify, and characterize the new compound. Screening can also be extended beyond antimicrobials (e.g., to include antimalarial, anticholesterol or anticancer activity); all of these diseases are commonly treated with drugs derived from natural products and thus amenable to the same SB approach.)
5. Repeat this procedure many times, at high throughput, to increase the chances that clinically valuable compounds are detected.
6. Transfer the biosynthetic machinery for compounds of interest into pre-designed, industrially validated, and safe production hosts, minimizing the risk that active, valuable compounds drop out late in the pipeline, because of limitations in production efficiency.
7. As the genes for the entire biosynthetic machinery are produced synthetically, it is also possible to try out different variants: antibiotics are typically produced by large assembly lines of enzymes, and it is possible to use SB to replace individual units or modules of these assembly lines systematically, so that instead of a single new compound, we obtain a library of slightly different variants, perhaps with better pharmacokinetics or broader activities.

Of course, like any emerging game-changing technology, SB raises a number of potential issues that could limit its industrial application. However, in its application to antibiotics discovery, these barriers are far less problematic than in many other fields. First, the host organisms for antibiotic production are well-characterized microbes that are generally regarded as safe, limiting ethical concerns associated with the creation of new life forms (e.g., compared to the manipulation of eukaryotic cells, higher animals, or security-relevant microorganisms). Second, drug discovery and production takes place in fermentation tanks, limiting security concerns about the release and containment of genetically modified organisms (e.g., compared to agricultural applications). Third, antibiotic production, and the general biosynthetic production of high-value compounds (e.g., drugs, food and

cosmetic additives, refined chemicals), is a highly efficient process, limiting economic concerns about resource competition (e.g., compared to biofuel production by SB).

Policy issues

SB approaches to drug discovery raise a number of important policy challenges:

- **Develop new funding mechanisms:** SB requires funding mechanisms that reflect its interdisciplinary approach, bringing together classical microbiology, natural products chemistry, and modern engineering and computational technologies. Examples include collaborative grants from the same or different institutions; inter-agency/cross-council funding initiatives; interdisciplinary SB training (discipline-hopping grants at various levels, support for initiatives like the International Genetically Engineered Machine competition [iGEM]); and interdisciplinary funding committees.
- **Stimulate more international collaboration:** This is essential, as the necessary expertise is sparse and often available only in single geographic areas (e.g., culture collections of exotic antimicrobial-producing bacteria and fungi). Cross-country funding initiatives are needed (e.g., the European Research Area grants on SB). Multilateral initiatives and funding from a single joint pot are strongly preferred over bilateral approaches and separate funding by country. As much as possible, opportunities should be created for research partners to be chosen by scientific quality, not by geography.
- **Fund high-risk fundamental research:** SB is a new field, and ambitious innovative projects are crucial. There is a clear danger that funding is targeted at close-to-market, application-ready projects, while the true benefit for the long-term solution of the antimicrobial drug discovery issue would come from high-risk fundamental research that establishes novel tools and concepts. A counterintuitive, but scientifically and economically desirable move would be to stop all funding for applied research in SB. Instead, increase the incentive for creative basic research that can become the basis for a new industrial revolution (e.g., by funding 10 SB proposals per year which are completely fundamental, out-of-the-box and very risky). These proposals should be funded without the need for publications or proof-of-concept data, selected based on scientific creativity and vision, and funded long-term (10 years).
- **Translate new technologies to industry:** Close industry-academia ties are essential, but instead of being focused on doing applied research for immediate industrial deployment, the aim should be to enhance industry awareness of new technologies. Create technology centers associated with a critical mass of state-of-art academic research, with the explicit mission of identifying industrial opportunities and translating the novel technology toward commercialisation (e.g., the National Graphene Institute at the University of Manchester).
- **Establish initiatives targeted at less-affluent markets:** Given the economic difficulties of developing new antimicrobials in a commercial setting, creative public and/or charitable initiatives are needed, along the lines established in the development of innovative drugs for less-affluent markets. Training in SB for less-affluent countries is needed, as are specific funding initiatives for the identification of SB targets to address specific needs in the developing world. This can go far beyond antibiotics and other drugs, but these are a good starting point, given that many infectious diseases are closely associated with poverty, and SB offers economically and environmentally attractive new routes to drug production.

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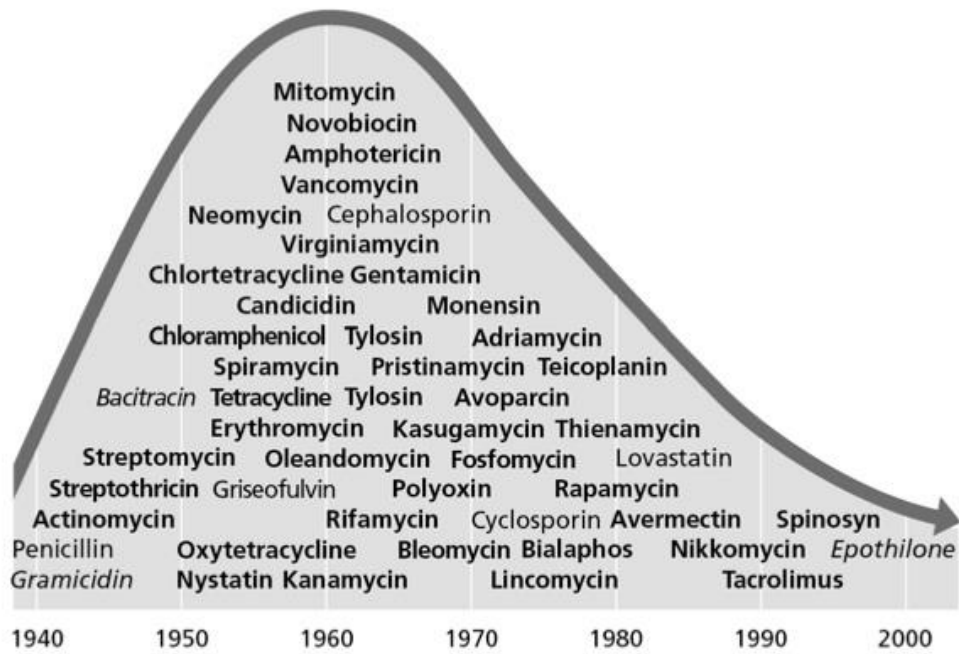
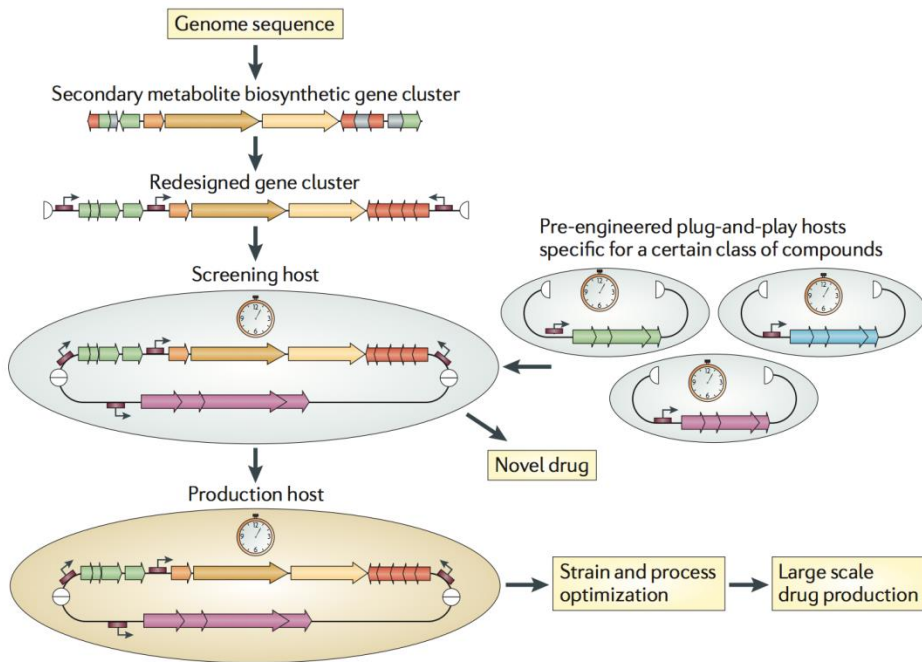


Figure 1A (Courtesy of David Hopwood, UK)



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Figure 1B (from Medema, Breitling, Bovenberg, and Takano, *Nature Reviews Microbiology*, 2011, 9:131-137)