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Editorial overview: Antimicrobials: Grappling with the complexities of antibiotics and resistance

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The increasing incidence of antibiotic-resistant infections coupled with a declining antibiotic pipeline is creating a global public health threat. Since conventional antibiotic discovery has failed to keep pace with the rise of resistance, novel approaches and rejuvenated efforts are needed to address this growing crisis. In this issue, the case is made that our antibiotic arsenal can be significantly enhanced by better understanding the actions of existing antibiotics and exploiting the bacterial responses induced by antibiotics and other lethal stresses.

Resistance to antibiotics is acquired either vertically through *de novo* mutations, or horizontally through transmission of resistance-conferring genes. Vertical evolution is considered in depth by Hartl, who describes our current view of adaptive 'fitness landscapes'. These landscapes are based on comprehensive measurements of resistance not only of individual mutations, but also of the entire space of their combinatorial combinations. Such measurements reveal the synergistic and antagonistic interactions among resistant mutations and their constraints on evolutionary adaptive pathways. The author concludes with pointing to a set of open questions at the interface of genetics, evolution and clinical applications, that can now be tackled within the framework of fitness landscapes.

Considering evolution of resistance through horizontal transfer, Wright and colleagues describe our current understanding of the resistome — the collective of genes in the environment that serves as a pool from which resistance can evolve. These genes have existed in nature long before the introduction of antibiotics and include silent and protoresistance genes, which serve as a source for the evolution of active resistance. Understanding this natural reservoir of resistance or protoresistance genes is important for predicting and inhibiting the evolution of antibiotic resistance in clinical settings.

Diversity of mechanisms appears not only in resistance but also in the mode of action of drugs. While many antibiotic discovery efforts focus on single-target screens, dirty drugs and prodrugs can offer new avenues for antimicrobials. Gopal and Dick call for re-analyzing and re-investing in reactive dirty fragments, old antimycobacterials that have been used to treat tuberculosis (TB). These drugs consist of small fragments that become active once metabolized by the microbe and subsequently hit multiple targets. The authors highlight pyrazinamide (PZA), a metabolized fragment drug that is used to prevent relapses in TB patients, and note that while some aspects of PZA's mode of action have been worked out, much remains unknown. The case is made that renewed efforts on fragment-based drugs

could reveal novel targets for antimycobacterial drug discovery, and significantly reduce treatment times and improve relapse rates for TB patients.

Even antibiotics that inhibit or corrupt single targets can induce complex physiological responses in bacteria. Zhao and Drlica review evidence showing that reactive oxygen species (ROS) play a role in the killing actions of antibiotics, but note that the situation is more complicated than originally proposed. In particular, it is argued that the induced ROS have a dual functionality depending upon the treatment condition — specifically, ROS play a protective role at low antibiotic stress and a lethal role at high drug concentrations. The authors highlight the genetic control elements involved in the response, and emphasize the need to utilize appropriate assays to characterize antibiotic-induced effects and distinguish between direct drug-related damage from the physiological responses induced by such damage. It is speculated that antioxidant dietary supplements may compromise the efficacy of antibiotic treatment by interfering with the oxidative stress components of antibiotic lethality.

The broad physiological effects of antibiotics on microbial cells provide unexpected ways by which bacteria can protect themselves against lethal stimuli. Luhachack and Nudler review the emerging body of work showing that bacterial gastrotransmitters can play an important role in the defense against antibiotics. Gastrotransmitters are small gaseous signaling molecules that can freely diffuse in and out of bacteria. The authors offer compelling evidence that two bacterial gastrotransmitters, nitric oxide and hydrogen sulfide, provide protection against oxidative stress and diverse classes of antibiotics, in part, by inducing ROS scavenging activity and inhibiting Fenton chemistry. It is argued that the associated pathways could be targeted as a means to boost the efficacy of currently available antibiotics and overcome resistance in some instances.

Antibiotic resistance is not only a single-cell phenomenon. Resistance to drugs and the production of small molecules mediate interactions among microbial populations. Kerr and colleagues show how the dynamics of these competing populations can be understood in terms of game-theory models. These models account for cheating and cooperation in antibiotic degradation and production. The authors stress that the outcomes of these competitions are fundamentally different in well-mixed and structured environments. They further suggest that these game-theory models can be extended to describe more complex scenarios of larger numbers of players and density-dependent regulation of antibiotic production.

The cooperation among cells that allows a greater protection against drugs is considered by Vega and Gore, who describe three principal ways in which such 'collective resistance' can emerge. First, individual cells may cooperate in degrading the antibiotic thereby generating a density-dependent resistance, known as the 'inoculum effect'. Second, cells organized into biofilms can slow down the diffusion of drugs and provide a spatial structure that allows the emergence of niches and functional diversification. Finally, bet-hedging strategies can generate phenotypic diversity through stochastic differentiation, allowing slower-growing 'persister' cells to survive the drug and regenerate the entire population once the drug is removed.

While from the population level bacterial persistence can be viewed as an adaptive behavior, Levin *et al.* propose that persisters could result more simply from physiological errors. Bacterial persisters are quasi-dormant cells that tolerate antibiotic treatment, and have been implicated in biofilms and in chronic and recurrent infections. The authors propose that persisters arise as a result of different types of errors or mishaps in cell physiology, and that the many genes identified as contributing to persister formation likely modulate the rate at which these errors occur. According to this provoking paradigm, antibiotic stress can contribute to the generation of persisters by increasing the rate at which these errors are produced. It is further argued that we need to better understand factors that modulate persister production if we are to develop effective options for treating persistent bacterial infections, including biofilm-based infections.

While persistence provides bacteria with a means to protect against drugs, toxin–antitoxin systems thought to be involved in persister formation could be exploited to kill the bacterial cells from within. Indeed, toxin–antitoxin systems have long been considered attractive candidates for antimicrobial agents, and Kumar and Engelberg-Kulka describe the identification of naturally occurring activators of these systems. Six species of peptides produced by different bacterial strains were found to enhance the RNase activity of both *Escherichia coli* and *Bacillus subtilis* toxin–antitoxin systems *in vitro* and thereby kill such bacteria in the lab. These discoveries open the door to the development of new types of drugs that kill bacteria by activating lethal pathways, rather than by inhibiting essential processes.

Together, these reviews provide a window into some of the novel ways by which we might be able to make progress in understanding antibiotic resistance in nature, and predict and possibly slow down the evolution of antibiotic resistance in the clinic.