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Current Opinion in Biotechnology

Toward a quantitative understanding of antibiotic resistance evolution

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The rising prevalence of antibiotic resistant bacteria is an increasingly serious public health challenge. To address this problem, recent work ranging from clinical studies to theoretical modeling has provided valuable insights into the mechanisms of resistance, its emergence and spread, and ways to counteract it. A deeper understanding of the underlying dynamics of resistance evolution will require a combination of experimental and theoretical expertise from different disciplines and new technology for studying evolution in the laboratory. Here, we review recent advances in the quantitative understanding of the mechanisms and evolution of antibiotic resistance. We focus on key theoretical concepts and new technology that enables well-controlled experiments. We further highlight key challenges that can be met in the near future to ultimately develop effective strategies for combating resistance.

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Current Opinion in Biotechnology 2017, **46**:90–97

This review comes from a themed issue on **Systems biology**

Edited by **Matthias Heinemann** and **Yitzhak Pilpel**

For a complete overview see the Issue and the Editorial

Available online 11th March 2017

http://dx.doi.org/10.1016/j.copbio.2017.02.013

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Introduction

Progress in our quantitative understanding of the evolutionary dynamics leading to antibiotic resistance holds promise to help avert the looming resistance crisis [1]. While changes in antibiotic prescription strategies can contribute to countering resistance [2], optimized treatment schemes that take into account the dynamics of resistance evolution are urgently needed. The best-known mechanisms of antibiotic resistance commonly found in the clinic or laboratory include antibiotic degrading enzymes, drug target modification, efflux, and the prevention of drug uptake [3–6]. These mechanisms have been characterized in great detail in decades of fruitful work, culminating in databases of the 'resistome'—the collection of all known genes conferring resistance [7–9]. More recently, transcriptomic studies have provided a useful intermediate phenotypic description of resistance, showing that information on global gene expression can improve predictions of the resistance phenotype compared to genotypic data alone [10,11].

The resistance of a bacterium to a drug is determined by measuring the minimal inhibitory concentration (MIC), that is, the lowest concentration that completely inhibits growth of a clonal culture [12]. An increase in resistance occurs when the population can grow in higher concentrations of antibiotic. Resistance is a genetically inherited trait, acquired by bacteria through one of two main processes: spontaneous *de novo* mutations and horizontal gene transfer [13,14]. Still, the level of resistance is often not entirely determined genetically, but can be heterogeneous within a population, depend on the environment, on the population structure, or on the physiological state of the cell [15–18]. In this review, we focus on specific examples where population dynamics and cell physiology affect drug sensitivity, and on quantitative aspects that determine the emergence and spread of *de novo* resistance mutations. We particularly emphasize recent studies that combined experiments and theoretical modeling. Related influential studies on collective resistance and on the effect of drug combinations on resistance evolution have been reviewed elsewhere [19–23].

Role of cell physiology and population effects in resistance

The growth rate of a bacterium depends on the nutrient environment and is a key physiological parameter that can strongly affect its sensitivity to a wide range of antibiotics [15,24••,25,26]. A recent experimental-theoretical study focused on ribosome-binding antibiotics, and showed that a lower growth rate (achieved by different growth media) increases the tolerated antibiotic concentration while for others, the opposite effect occurs [24••]. A mathematical model based on bacterial 'growth laws' [27], which take into account how the ribosome concentration in the cell depends on growth rate, showed that the ribosome-binding kinetics of the drug can explain this effect: slow-growing cells are more resistant to reversibly binding drugs, whereas fast-growing cells are more resistant to irreversibly binding drugs [24••]. These results would have been hard to intuit without using a rigorous theoretical approach and highlight that apart from specific molecular mechanisms, global cell physiology and growth rate are important determinants of antibiotic resistance levels.

Glossary
MIC: Minimal inhibitory concentration. The lowest concentration of an antibiotic that completely inhibits growth of a clonal culture.
TEM-1 β-lactamase: An enzyme produced by bacteria that cleaves and deactivates β-lactam antibiotics.
DFE: Distribution of fitness effects. The probability distribution that represents the changes in fitness caused by single-step mutations originating from a common genotype. It depends on the ancestral genotype and on the environment.
Epistasis: The phenomenon that the effect of a mutation depends on the genetic background it occurs in.
Discrete fitness landscape: A graph in which the vertices are genotypes, each with an assigned fitness value. Two genotypes are connected by an edge if they are a single mutational event apart.

Global cell physiology can even explain how a clonal population diversifies into growing and non-growing cells in the presence of antibiotics. The expression of many genes increases with increasing growth rate [28]; this effect alone can lead to bistable population dynamics [29]. Specifically, it was shown that in an *Escherichia coli* strain that expresses the cat1 enzyme, which inactivates the antibiotic chloramphenicol (Table 1), a positive feedback loop occurs where a decrease in growth rate due to addition of more chloramphenicol decreases expression of the resistance-conferring enzyme, thus slowing growth even further [30]. Theory shows that such a positive feedback loop can lead to bistability, that is, coexistence of growing and non-growing cells at the same drug concentration; this striking effect was confirmed in single cell experiments [30]. Growth bistability is likely a more general phenomenon [31] that occurs for other resistance mechanisms and highlights that the response of a population of clonal bacteria to antibiotics is not simply given by many identical copies of the same cell.

Population effects are also important when resistance is due to extracellular antibiotic degradation. Here, the antibiotic concentration in the medium strongly depends on the cell density, since higher densities lead to faster antibiotic degradation. The inoculum size of the culture thus affects the growth of all cells and, ultimately, the measured resistance level. This effect has been described in mathematical models and experimentally validated using the beta-lactamase enzyme which degrades beta-lactam antibiotics (including amoxicillin, ampicillin, and cefotaxime) [32,33]. Such effects generally occur whenever a resistant subpopulation degrades or modifies the antibiotic so that the entire population can benefit from it. It will be interesting to further investigate the causes and consequences of these effects which also occur for other antibiotics [34].

Table 1

Glossary of antibiotics and their targets

Antibiotic	Target
Chloramphenicol	50S ribosomal subunit
Tetracycline	30S ribosomal subunit
Amoxicillin	Cell wall synthesis
Trimethoprim	Folate synthesis (DHFR)
Ciprofloxacin	DNA replication (DNA gyrase)

Studying antibiotic resistance using experimental evolution

Beyond characterizing existing resistance mechanisms, it is a fundamental question how *de novo* resistance evolves. Understanding this can ultimately lead to strategies for inhibiting resistance evolution. Recent years have seen a plethora of novel techniques for investigating antibiotic resistance evolution in the laboratory and for systematically addressing its reproducibility, speed, molecular origins, and constraints.

Resistance often evolves so fast that it can be studied in the laboratory but it is still challenging to obtain quantitative and reproducible results. Serial transfer of microbial cultures is a common experimental evolution protocol [35] that is also useful for studying resistance evolution [22,36,37]. In this protocol, bacterial cultures grow in flasks or on microtiter plates and are diluted into fresh medium by a fixed factor at regular time intervals (*e.g.*, every 24 hours). These experiments can be continued virtually indefinitely: Richard Lenski's seminal long-term evolution experiment [35] has exceeded a staggering 60 thousand generations in 28 years and is still ongoing. Because of the relative simplicity of this protocol, it is feasible to run hundreds of evolution experiments in parallel. Together with increasingly inexpensive whole genome sequencing techniques [38], this opens the door for a statistical investigation of the intrinsically stochastic evolutionary dynamics and for identifying general principles governing microbial evolution [39–42]. A drawback of serial transfer protocols is their inability to keep key parameters that affect the evolutionary process well-controlled: the population size fluctuates considerably and cultures differ in their growth rates and in the time they spend in stationary phase. This complicates the quantitative investigation of the evolutionary process and its comparison among different cultures. Furthermore, it is not straightforward how the antibiotic concentration should be chosen in such experiments to gain maximum insight into the process of resistance evolution: if it is too low, there is virtually no selection for resistance; if it is too high, cells cannot grow at all, preventing them from evolving at a significant rate.

Recently developed techniques in which bacteria are exposed to increasing antibiotic concentrations solve this problem. Theoretical work suggested that temporal or spatial selection gradients can facilitate the sequential emergence and fixation of multiple resistance mutations leading to increasingly higher resistance levels [43,44]. Consequently, advanced protocols that gradually increase antibiotic concentrations in time or space have been

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