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Toward prediction and control of antibiotic-resistance evolution

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The emergence of antibiotic-resistant bacteria is a serious public concern. To deal with this problem, recent advances in technology and the use of laboratory evolution experiments have provided valuable information on the phenotypic and genotypic changes that occur during the evolution of resistance. These studies have demonstrated the existence of evolutionary constraints on the development of drug-resistance, which suggests predictability in its evolution. In this review, we focus on the possibility to predict and control the evolution of antibiotic resistance, based on quantitative analysis of phenotypic and genotypic changes observed in bacterial laboratory evolution. We emphasize the key challenges in evolutionary biology that will contribute to the development of appropriate treatment strategies for preventing resistance evolution.

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Introduction

The emergence of multidrug-resistant bacteria is an increasing public health concern worldwide [1,2]. Clinical doses of antibiotics offer selective benefits to naturally occurring resistant bacteria, resulting in the evolutionary dynamics of antibiotic resistance [3,4]. Establishing strategies to suppress the emergence of resistant bacteria is an urgent task; therefore, further understanding of the evolution of drug resistance is needed.

Antibiotic resistances are acquired either through mutations or by horizontal gene transfer of resistance-related genes [5–7]. With the advancement of experimental technologies, particularly with the development of high-throughput sequencing, genetic alternations have been identified in clinically and experimentally obtained resistant strains, which provide valuable information on the dynamics of resistance evolution [8–10]. For some of these genetic changes, one can easily extract the causal relationship between mutation and resistance acquisition, such as those related to drug degradation, modification of the drug target, or activation of a drug efflux pump. However, the relationship between a genetic change and drug resistance is not always simple. The effects of mutations on drug resistance are not always simply 'additive,' and in fact, non-trivial epistatic interactions among mutations are ubiquitous [11,12]. Due to such complex interactions, the relationships among drug resistance acquisition, genetic alternations, and global phenotypic changes appear to be complex and remain elusive.

On the other hand, the complexity of antibiotic evolution may provide clues allowing drug evolution to be predicted and controlled. The interactions between genetic changes and resistance mechanisms often produce restrictions on the possible evolutionary courses for antibiotic resistance [13–15]. In fact, results of laboratory evolution experiments demonstrate that bacterial evolution is often repeatable, which is consistent with limitations in possible evolutionary courses [8,16,17]. This suggests that by understanding such constraints, we may be able to predict the evolutionary outcome, and thus potentially control it by adding designed environmental conditions, such as the use of appropriate drug combinations, or dynamically changing drug usage. In this review, we focus on the possibility of predicting and controlling the evolution of antibiotic resistance, based on quantitative analysis of phenotypic and genotypic changes observed in bacterial laboratory evolution.

Laboratory evolution of antibiotic resistance

Laboratory evolution is a powerful tool to quantitatively analyze phenotypic and genotypic changes in bacterial evolution [18,19]. Serial transfer of cultures is a popular bacterial laboratory evolution protocol, in which bacterial cultures are grown in flasks or on microtiter plates, and are diluted and transferred into fresh medium at constant time intervals. A pioneering study of long-term laboratory evolution by Lenski and his colleagues [20,21,22••] has maintained *E. coli* populations for more than 6×10^4 generations. The study is ongoing and has provided valuable information on the dynamics of evolutionary adaptation and its genetic basis.

Laboratory evolution experiments using increasing antibiotic concentrations for studying the evolution of antibiotic resistance is widely done as an alternative to the serial transfer of cultures within a fixed environment [23,24••,25]. One standard protocol is to inoculate cells into environments with different drug concentrations, and at constant time intervals, the cells are cultured in the highest drug concentration possible in which the cells can sustain growth. Through iteration of this propagation, a gradual increase develops in the minimal inhibitory concentration (MIC), the lowest concentration of drug that completely inhibits cellular growth. An advantage of this method is the ability to perform, in parallel, high-throughput laboratory evolution in 96-well and 384-well microtiter plates. Another alternative protocol is called 'morbidostat,' which is a system with dynamic feedback regulation of the drug concentration [8,26]. This is a continuous culture system in which the drug concentration is increased depending on the cell concentration and allows for strong selection pressure for resistance to be constantly maintained, resulting in reproducible courses in the evolution of antibiotic resistance.

Laboratory evolution using a spatial gradient of antibiotic concentration has also been studied intensively [27,28••]. For example, antibiotic-resistance evolution was analyzed using a drug gradient maintained on a large 120 cm × 60 cm agar plate called the microbial evolution growth arena, or MEGA plate [28••]. On this agar plate, cells spread by chemotaxis, and the dynamics of the spatiotemporal evolution of antibiotic resistance is visualized in a Muller diagram (e.g., Figure 3 in [29]).

Prediction of antibiotic-resistance evolution

Whether evolutionary dynamics are predictable or not is a subject of long-standing debate in biology [15,30,31]. Evolution inherently involves randomness, which implies that the evolutionary dynamics are unpredictable. However, recent studies demonstrate convergent evolution at the phenotype and genotype levels, that is, a similar outcome may evolve in independent experiments [14,32]. This observed repeatability of evolutionary dynamics suggests the existence of constraints in evolution, which in turn suggests their predictability.

One cause of the constraints or restrictions in evolutionary dynamics is the interactions among the genetic alternations. The fitness effect of a mutation depends in part on the genetic background, including other mutations. This phenomenon, called epistasis, can make the fitness landscape complex and rugged, resulting in constraints on the possible evolutionary pathways [13,33]. A well-known example is mutations in TEM-1 β-lactamase, which provides resistance to β-lactam antibiotics [34]. Various studies, based both on clinical isolates and *in vitro* analyses, indicate that the effects of multiple mutations in the β-lactamase gene may not always be additive. Rather, epistasis among these mutations results in a complex fitness landscape and constrains the evolutionary pathways [35]. For example, it is known that five mutations in a particular β-lactamase gene jointly increase resistance to the β-lactam antibiotic cefotaxime by a factor of ~100 000-fold [36]. To elucidate the evolutionary pathways leading to this high resistance, various combinations of these five mutations were introduced into the β-lactamase gene and their effects on drug resistance quantified [37–39]. These analyses suggest that only part of the mutational trajectories is likely to be realized during selection for β-lactam resistance, owing to the epistatic interactions among the mutations [36]. This constraint on possible evolutionary pathways may be responsible for the repeatability and predictability of protein evolution [17].

In addition to enzyme evolution, the constraints of evolutionary pathways are also observed in various phenotypic changes. Well-known examples of this are collateral resistance and sensitivity during the evolution of antibiotic resistance, which are the phenomena of acquisition of resistance to one drug being accompanied by the concomitant acquisition of resistance or sensitivity to another drug [23,40,41]. For example, the acquisition of resistance to protein synthesis inhibitors aminoglycosides in *E. coli* is accompanied by collateral sensitivity to other antibiotics such as tetracycline and chloramphenicol. This collateral sensitivity associated with resistance acquisitions is suggested to be caused by changes in the proton-motive force (PMF) across the cell membrane [40], which constrains the possible course of drug-resistance evolution in *E. coli*. In other words, the *E. coli* cells cannot acquire resistances to both drugs simultaneously. This collateral resistance and sensitivity form a complex network among drugs, which may affect the course of resistance evolution. Evolutionary constraint related to metabolic balance has also been demonstrated recently for the evolution of drug resistance in *E. coli* [24••].

Limitations in the drug-resistance profiles are likely reflected in the low-dimensional nature of phenotypic changes. In fact, transcriptome analysis of laboratory-evolved strains of *E. coli*, resistant to various antibiotics with different mechanisms of action, shows that the level of resistance can be quantitatively predicted based on the expression levels of a small number of genes (e.g., 7 or 8), using a simple mathematical model [41]. This suggests that the change in the gene-expression profile during resistance evolution is relatively restricted by low-dimensional dynamics, which results in predictable antibiotic resistance. It should be noted that recent experimental and theoretical studies demonstrate that possible phenotypic changes during adaptation and evolution may be constrained by low-dimensional dynamics [42–45]. Unveiling the low-dimensional nature of phenotypic changes in resistance evolution might contribute to