What counters antibiotic resistance in nature?

Remy Chait, Kalin Vetsigian & Roy Kishony

Antibiotics promote the spread of resistance in the clinic, but various mechanisms may exist in natural environments that tilt the balance toward antibiotic sensitivity. Studying such mechanisms could help us understand the evolutionary dynamics of resistance and sensitivity in the wild, which may inspire new therapeutic strategies.

Antibiotics, which permit the effective treatment of bacterial infections, represent a fundamental triumph of medical science. However, their widespread clinical and agricultural use has led to the rapid emergence and spread of resistance. This proliferation of resistance reduces or eliminates the utility of antibiotics, rendering some clinical infections dangerously untreatable[1]. In light of the slow rate of discovery of new drugs, reducing the rate at which resistance evolves offers a tenable strategy for maintaining the efficacy of existing and future antibiotics[1,2].

To gain some insight into mechanisms that can keep antibiotic resistance in check, it is interesting to consider an evolutionary-ecological perspective. Most clinical antibiotics are derivatives of natural microbial products that evolved long before the era of medicinal antibiotic use and are commonly found in the chemical repertoire of soil microbes[3–5]. Resistance to these compounds evolved in the natural environment long ago, and in fact the resistance genes we see in the clinic today often bear similarity to genes in the environment[3,6,7]. Although the compounds and the resistance genes are similar in these two contexts, their dynamics are profoundly different. Whereas clinical resistance tends to increase substantially with time, resistant and sensitive bacteria have presumably existed together in soil environments for much longer periods (**Fig. 1**). Why is it that resistant bacteria do not take over in the soil environment? What mechanisms might be acting in nature to keep antibiotic resistance in check? We discuss several aspects of the soil environment that could explain why antibiotic resistance does not take over, including antibiotic combinations, compounds that inhibit or toxify resistance, degradation products of antibiotics and other ecological roles of antibiotics.

Antibiotic combinations

One striking difference between the clinical and soil environments is that microbes in the clinical context are exposed to one (or few) drugs at a time, whereas those in a soil environment are likely to encounter many more toxins simultaneously. Can the mere presence of multiple drugs cause selection against resistant bacteria? Recently, we investigated the advantage of tetracycline resistance in cells that were exposed to tetracycline combined with other antibiotics. In particular, we examined the effect of synergistic combinations, in which the combined effect of both drugs together is greater than expected, and of suppressive combinations, in which the effect of both drugs is weaker than that of one of the drugs alone[8,9]. We found that in the synergistic case, resistance eliminated not only inhibition due to a tetracycline

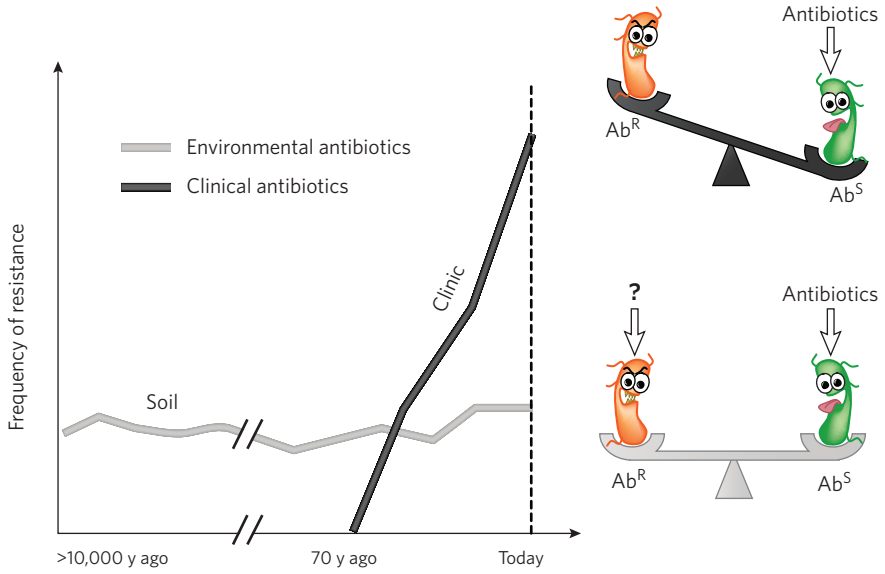

Figure 1 | The dynamics of antibiotic resistance in clinical settings may profoundly differ from that in the natural environment (schematic illustration). High doses of antibiotics administered in the clinic since the mid-twentieth century have overwhelmed other forces and led to fast, dramatic increases in the prevalence of antibiotic-resistant (Ab^R) versus antibiotic-sensitive (Ab^S) pathogens (black line in graph and balance at top right). In contrast, antibiotic resistance and sensitivity have existed together in soil microbial environments since long before human antibiotic discovery and use, suggesting that other factors may balance selection by antibiotics naturally present in the environment (gray line in graph and balance at bottom right).

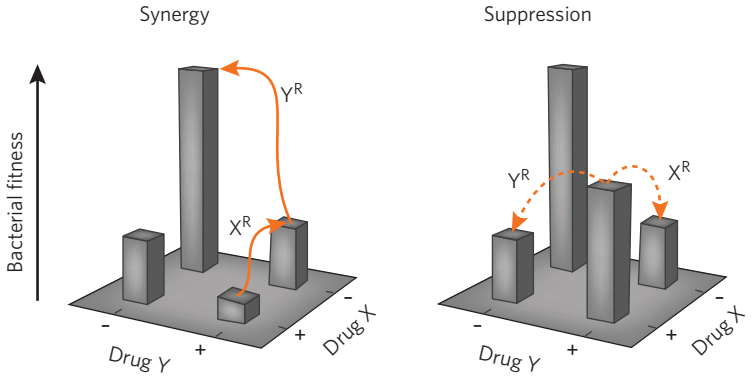

Figure 2 | Synergistic and suppressive antibiotic combinations respectively enhance and invert selection for resistance to their components. Resistance to one antibiotic in a synergistic drug pair reduces inhibition by the compound as well as that due to synergy, resulting in a large increase in the fitness of the resistant strain (left, X^R). Consequently, the resistant mutant can outgrow the sensitive strain and is free to acquire a second mutation, conferring resistance to the entire combination (Y^R). In contrast, the inhibition alleviated by resistance to a compound in a suppressive combination is outweighed by the loss of its protection from the second drug, leading to a net decrease in fitness due to resistance and blocking its spread.

but also inhibition due to the synergistic combination, thus giving a greater-than-expected advantage to the resistant strain (**Fig. 2**). However, in the suppressive combination, eliminating the inhibition caused by a tetracycline also removes the tetracycline's partial suppression of the second drug's effect, resulting in a net decrease in the fitness of the resistant bacteria (**Fig. 2**). Such selection, which favors sensitivity resulting from suppressive combinations of antibiotics, is largely independent of molecular mechanisms of resistance and can occur at concentrations of tetracycline below the minimum inhibitory concentration[8]. Though the frequency of suppressive interactions between toxins in the natural environment is difficult to assess, a survey of pairwise interactions between 21 different antibiotics (many of them natural or derived from natural products) found roughly 10% to be suppressive *in vitro*[10]. In a related phenomenon, selection that favors sensitivity over resistance is also observed when bacterial populations are transiently inhibited by bacteriostatic (growth-arresting) agents while exposed to conditions that kill only growing bacteria. For instance, though inhibitory concentrations of tetracycline or sulfonamide halt the growth of sensitive bacteria and thereby protect them from being killed by penicillin, strains that are resistant to tetracycline or sulfonamide continue to grow and are rapidly dispatched by penicillin[11]. This phenomenon is equally difficult to quantify in the wild, especially because even nonchemical stresses such as predation by bacteriophages can serve as the bactericidal component[12]. Both systems, however, clearly illustrate the potential for evolutionary selection in favor of antibiotic sensitivity over resistance to emerge from the simultaneous occurrence of multiple stresses in natural environments.

Inhibiting and toxifying resistance mechanisms

Selection by combinations need not stem purely from interactions between toxins. Indeed, by combining antibiotics with compounds that neutralize resistance mechanisms (for example, by inhibiting antibiotic-degrading enzymes or by blocking antibiotic efflux pumps), the efficacy of the drug can be restored and the selective advantage of resistance can be removed. In fact, β-lactams combined with β-lactamase inhibitors (for example, amoxicillin with clavulanic acid) have proven to be very effective in the clinic. It is interesting to note that certain soil microbes that produce β-lactam antibiotics also produce such inhibitors of β-lactamases, raising the question of whether these microbes evolved to control the evolution of resistance and protect the efficacy of their toxins[13].

Selection against resistance may even be imposed by individual toxins or stresses. Just as resistance to one antibiotic can protect against another ('cross-resistance'), various mechanisms of resistance may increase sensitivity to certain toxins (termed 'negative cross-resistance' or 'collateral sensitivity')[14–16]. Thus, although positive cross-resistance to toxins such as heavy metals can select for antibiotic-resistant strains in the absence of antibiotics[6], negative cross-resistance to elements of the environment may instead favor sensitivity and limit the spread of resistance. For instance, soil-isolate production of compounds such as fusaric acid, which antagonizes tetracycline resistance mediated by the tetA efflux pump, suggests an additional means by which microbes could control levels of resistance to antibiotics in the wild[15,17].

Antibiotic degradation products

A particularly interesting form of negative cross-resistance occurs when resistance to an antibiotic is counteracted by the products of the antibiotic's chemical decay (**Fig. 3**). For instance, as tetracycline decays into anhydrotetracycline, selection in favor of tetA-mediated tetracycline resistance is not only diminished but in fact inverted to favor tetracycline sensitivity[18]. In such cases, an antibiotic may serve a dual role for its producer, initially inhibiting the growth of sensitive strains while selecting for resistance and subsequently maintaining susceptibility by selecting against resistance as the antibiotic degrades. Estimates of the overall effect of any compound in the environment should take into account not only its immediate effect but also its degradation time and the effect of the compounds to which it degrades. It should be noted that although many antibiotics are unstable, those administered in the clinic are usually flushed from the body rapidly, whereas those produced in a soil context could persist and remain exposed to chemical degradation over longer periods. This distinction between clinical and natural antibiotic environments points to another mechanism by which the levels of antibiotic resistance could be controlled differently in the wild and in the clinic.

Screen for selective compounds

The compounds referred to above may represent just the tip of the iceberg in terms of microbially produced chemicals that can select against antibiotic resistance. To sample the rich diversity of natural microbial products in a broader and less directed manner, we devised a direct screen for compounds that bias selection against antibiotic resistance[16]. Briefly, fluorescently coded antibiotic-sensitive and antibiotic-resistant bacterial strains directly compete with each other in diffusing gradients of test compounds. Imaging in the strains' fluorescent channels quantifies their relative growth and identifies test compounds that select for sensitivity; they inhibit the resistant strain to a greater extent than the sensitive strain (**Fig. 4**). A small-scale pilot of the screen indicated that approximately

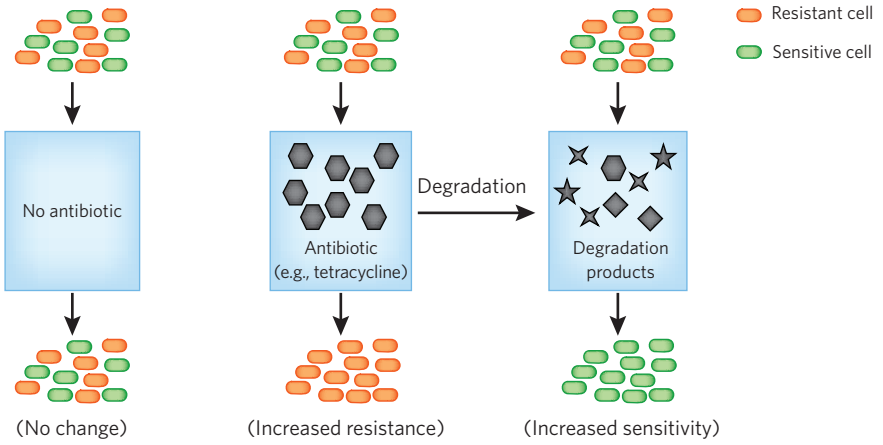

Figure 3 | The degradation products of antibiotics can select against resistance. In the absence of any drug (left), antibiotic-sensitive and antibiotic-resistant bacteria (green and red, respectively) grow together at similar rates and maintain a constant ratio in the overall bacterial population. The presence of an antibiotic (middle) permits the growth of the strain resistant to it while strongly impeding the growth of the sensitive strain, skewing the overall population toward resistance to the drug. As the antibiotic degrades (right), certain chemical degradation products favor the growth of the sensitive rather than the resistant strain and move the population toward increased sensitivity. (Adapted from ref. 18.)

1% of cultured soil microbes were secreting compounds that, alone or in combination with tetracycline, biased selection toward tetracycline sensitivity (**Fig. 4**)[16]. In contrast with more traditional tests for microbial growth inhibition, assays that measure differential growth inhibition generally provide a clearer window into the effects of compounds on evolutionary selection for and against antibiotic resistance.

Dynamic selection

Selection for or against resistance may be a highly dynamic process that varies over time and space. Unlike in the clinic, where the use of an antibiotic can be maintained even as it becomes less effective, in nature there is likely selection against producers of ineffective toxins owing to the metabolic cost of production. As toxin production decreases, resistance becomes unnecessary and is either selected against because of its cost or spontaneously lost through evolutionary drift[19]. The picture that we observe in the soil may represent a snapshot from a dynamic process that reflects the complex, evolving network of interactions between species[20].

Antibiotics as signals

Of course, though our experience with antibiotics in the lab and in the clinic relates primarily to their role as inhibitors of microbial growth, their precise role in nature remains unclear. Despite the prevalence of antibiotic production by wild microorganisms[4,5], few studies have shown that antibiotic-producing strains can benefit from the inhibitory function of their products in the wild[21–24]. Indeed, the possible role of these compounds as microbial toxins is challenged by their observed low concentrations in natural environments and their alternative molecular functions[24]. Coupled with the wide-ranging effects of subinhibitory concentrations of antibiotics on gene transcription and microbial behavior, such as swarming, virulence or biofilm formation, these observations have led to suggestions that the natural role of many of these compounds is not inhibitory but rather may be to act as signaling agents within microbial communities[23,25]. In such contexts, antibiotic resistance would still be expected to have substantial effects by modulating signal intensities or other noninhibitory antibiotic functions. Careful *in situ* experiments will assist in clarifying the degree to which this picture reflects the natural environment and in predicting specific situations that would benefit organisms that are sensitive or resistant to the antibiotic signals.

Outlook

Although certainly incomplete, this Commentary is intended to illustrate a few mechanisms that may be acting in nature to control the spread of antibiotic resistance over long periods of time. Actual selective forces on antibiotic resistance, production and sensitivity ultimately depend on the entire environment and the network of interactions in which species are embedded[23,24]. Though capturing the full chemical complexity of the environment is challenging, recent theoretical and experimental work has begun to examine community-wide statistical patterns of microbial interactions[20]. Such systematic studies should both inform and be supplemented by competition-based measurements of selection on antibiotic resistance and production, similar to those described above[16], that are conducted within raw microbial environments themselves and

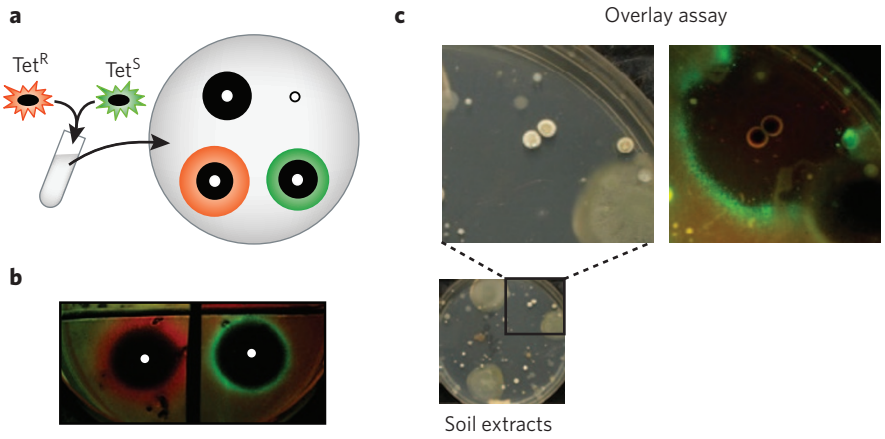

Figure 4 | Differential inhibition assay identifies products of soil microbes that exert selection for or against tetracycline resistance. (**a**) Agar diffusion assay for relative inhibition of mixed differentially labeled tetracycline-sensitive (Tet^S, green) and tetracycline-resistant (Tet^R, red) *Escherichia coli* by compounds diffusing from white dots. (**b**) On imaging, ratios between the fluorescently labeled strains reveal test compounds that, alone (not shown) or in combination, favor either the resistant strain (erythromycin + tetracycline, red ring) or the sensitive strain (ciprofloxacin + tetracycline, green ring). (**c**) Applying this assay to colonies isolated from soil shows microbes secreting substances that favor the growth of tetracycline-sensitive over tetracycline-resistant cells. (Adapted from ref. 16.)

are thus free from the confounding effects of the laboratory. It is hoped that in the near future, the combination of careful *in situ* experiments, measurements of community-wide microbial interactions and detailed characterization of the chemical actors therein will allow us to account more fully for the context of the natural environment and begin to tease out the major contributors to the coexistence of antibiotic-resistant and antibiotic-sensitive bacteria in the wild. ■

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Competing financial interests

The authors declare no competing financial interests.