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Microbial Ecology: Complex Bacterial Communities Reduce Selection for Antibiotic Resistance

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Competition between antibiotic-resistant and -susceptible bacteria is well studied in single-species communities, but less is known about selection for resistance in more complex ecologies. A new experiment shows natural microbial communities can hinder selection by increasing the fitness costs of resistance or by offering protection to drug-sensitive strains.

Antibiotics are a landmark achievement of 20th-century science. They represent a triumphant case study at the intersection of curiosity-driven discovery and society-changing practicality. It is no exaggeration to say that antibiotics revolutionized modern medicine. Countless lives were saved as previously debilitating infections became treatable, even curable. For a time, it seemed that the scourge of microbial infections had been bested, the once formidable pathogens vanquished not by synthetic chemistry alone, but with the help of molecular warfare pioneered by other microorganisms. Microbial communities that ravaged their mega-sized hosts could now be suppressed thanks to the chemical ingenuity of their own micron-scale neighbors.

Unfortunately, we now know that the successes of our antibiotic era are under threat [1]. Bacteria have proven to be remarkably agile tacticians, nimbly plotting a course through the genetic and phenotypic space of drug resistance. A drug that targets DNA replication? No problem — evolution identifies mutations in target helicases that reduce drug binding without dramatically sacrificing functionality. A small-molecule inhibitor of protein synthesis? There's a native efflux pump for that, lying in wait on the chromosome. Decades of rapid progress in molecular biology have identified a suite of antibiotic defenses, leading to an increasingly clear picture of the molecular determinants of resistance [2]. Unfortunately, these breakthroughs have not yet spawned 'resistance-proof' drugs, a goal that increasingly seems out of reach given the breadth and variety of resistance mechanisms. At the same time, as our understanding of the molecular-level dynamics matures, we're faced with a daunting collection of unanswered questions about how those microscopic details conspire to create large-scale community behavior at the level of cell populations. Are microbial communities simply amplified versions of single cells, an aggregate system where the whole is merely the sum of the parts? Or is community-level behavior — including the response to drugs — dominated instead by the intricate ways in which those cellular dynamics fit together? The answer to these questions — and a deeper understanding of the competition and cooperation that shape microbial societies — may hold clues for slowing, and even reversing, antibiotic resistance.

In a recent paper published in the *ISME Journal*, Klumper and colleagues [3] combine laboratory experiments in semi-natural microbial communities with mathematical modeling as a step toward addressing these questions. Studying complex communities is challenging; even with modern genomics tools, making sense of the exponentially growing number of potential inter-species interactions quickly becomes intractable. To overcome these inherent hurdles, Klumper *et al.* [3] devised a simple and powerful competition assay that focused on pairwise selection for resistance in *Escherichia coli* strains engineered to express an antibiotic-degrading enzyme along with a fluorescent marker. Competition among two species is easy to measure in a well-mixed population: one simply compares their growth over the fixed time period of interest — in this case, by counting fluorescent colonies in plated population samples. These proliferation rates, in turn, are converted to a relative fitness value (f) for the resistant strain: if sensitive and resistant cells grow equally well, the fitness approaches 1, whereas deviations indicate that growth of sensitive ($f < 1$) or resistant ($f > 1$) strains is favored. Klumper *et al*. [3] repeated this two-strain competition assay for a wide range of antibiotic concentrations, and consistent with previous findings [4], they observed a transition between two regimes, a low-concentration regime where sensitive strains are favored and a high-concentration regime that preferentially selects for resistance (Figure 1). In other words, the resistance comes with a cost — a collateral burden borne from heightened defenses (enzyme production) in a time of relative prosperity (low drug concentrations). But as conditions deteriorate with increasing drug concentrations, those costly defenses become indispensable, giving resistant strains a competitive advantage at concentrations that exceed a critical value known as the 'minimum selective concentration'. It's notable that the minimum selective concentration is considerably smaller than the 'minimum inhibitory concentration' — the cutoff concentration that completely stops growth of the sensitive cells [4]. Selection, it seems, does not require the drugs to overwhelm the sensitive cells, but begins precisely at the point

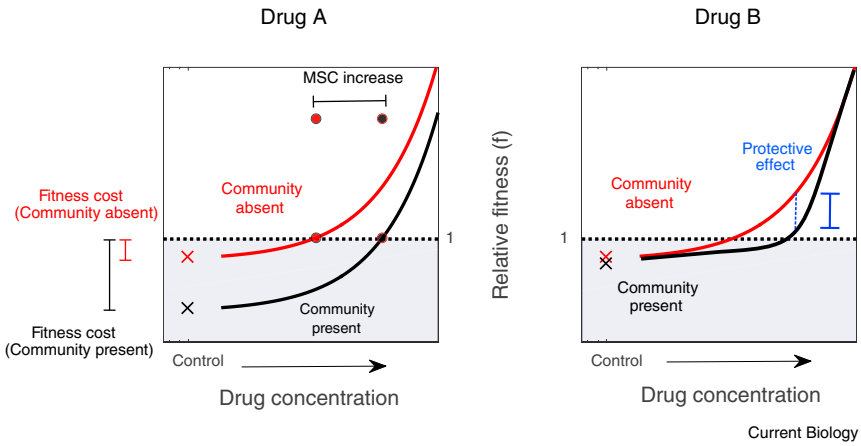

Figure 1. Semi-natural microbial communities modulate costs and benefits of antibiotic resistance.
Potential effects of microbial communities on selection for resistance to two different antibiotics, drug A (left) and drug B (right). Relative fitness (f) describes the competitive advantage (f > 1; white region) or disadvantage (f < 1; grey region) associated with the presence of antibiotic resistance — in this case, expression of a drug-degrading enzyme. In the absence of drug (Control), resistance typically comes with a cost (f < 1; red x, left panel), but increasing drug concentration leads to selection for resistance starting at a critical concentration, the minimal selective concentration (MSC; red circles, left panel). Klumper *et al.* [3] find that selection for resistance in the presence (black curves) or absence (red curves) of complex microbial communities can differ in several different ways (left panel versus right panel). The community may decrease selection by imposing an additional fitness cost of resistance (drug A, left panel), increasing the minimum selective concentration without altering the minimum inhibitory concentration for the sensitive strain. Alternatively, the community may provide a protective effect for sensitive cells, increasing their growth rate and effective minimum inhibitory concentration without modulating drug-free fitness costs (drug B, right panel). Protective effects are expected to diminish at low drug concentrations, at which inhibitory effects are minimal, and at high concentrations, at which they are insufficient to counter drug inhibition.

where subinhibitory concentrations render beneficial the otherwise burdensome molecular defenses. Sensitive cells need not be crippled to promote resistance; they simply need to be outpaced by their well-armed bacterial siblings.

The effects of subinhibitory antibiotic concentrations are well studied in single species communities, where they can lead to significant selection for resistance [4–7]. From a practical perspective, these results are unsettling, as they point to widespread selection for resistance even when drugs are present at low concentrations. At the same time, they seem to contradict observations from some natural microbial communities that show surprisingly little high-level resistance, despite the ubiquitous presence of antimicrobial compounds in the environment [8,9]. One potential explanation for this discrepancy is that the dynamics of selection in mixed communities may not follow, in a simple way, from the selection dynamics of isolated strains. Instead, sensitive and resistant cells — when coexisting — may interact in ways that spark fundamentally new dynamics. Indeed, a number of recent studies [10–12] demonstrate that the response of bacterial communities to antibiotics can be a collective phenomenon, giving rise to composition-dependent behavior where drug inhibition is modulated by (for example) enzyme expression [10,12], growth bistability [13], small molecule signaling [14], or changes to environmental variables such as pH [15].

It is increasingly clear that, when it comes to selection for resistance, context matters. But how can we scale the results from simple communities to systematically probe the enormous complexity of more realistic populations? Klumper *et al.* [3] used a clever approach: they incorporated semi-natural communities (specifically, communities derived from pig fecal extract) into selection experiments, but rather than tracking large-scale dynamics in potentially hundreds of species, they focused primarily on relative selection between sensitive and resistant 'focal stains' — the very same strains that were competing in the previous two-strain selection experiments. By comparing selection dynamics at different drug concentrations in the presence and absence of the complex community, the authors uncovered two qualitatively distinct community-driven effects (Figure 1) and, using simple mathematical models, proposed mesoscale ecological mechanisms to explain the dynamics. In the first case — selection for the antibiotic gentamicin — the community substantially decreased selection for resistance by imposing increased fitness costs on the resistant strain (Figure 1, left panel). The result was a significant increase in minimum *selective* concentration without a corresponding change in the minimum *inhibitory* concentration of the sensitive strain — an effect that could be reproduced in mathematical models by imposing strain-specific but concentration-independent competition between the community and the focal strains. Interestingly, the minimum selective concentration — initially much smaller than the minimum inhibitory concentration of the susceptible strain — increased in the presence of the community to concentrations approximating the minimum inhibitory concentration. The results suggest that competitive interactions in complex communities may partially explain the lack of high-level resistance in natural communities.

By contrast, the presence of the community did not generally increase fitness costs during experiments with the antibiotic kanamycin [3]. Instead, the relative fitness was largely unaffected by the community at both high and low drug concentrations, with a community-driven decrease in the relative fitness of resistant strains at intermediate concentrations (Figure 1, right panel). Numerical simulations imposing a community-mediated protective effect — implemented by dose-response parameters that depend

explicitly on cell density in the community — reproduced these observations. Consistent with a protective effect, the minimum inhibitory concentration of sensitive strains increased in the presence of the community. The minimum selective concentration was also increased — indicating that resistance is less favored in the community context — though in contrast to the gentamycin example, the minimum selective concentration for kanamycin remained far below the minimum inhibitory concentration of the sensitive strain.

The work by Klumper *et al.* [3] underscores the idea that ecological context can modulate the selection of resistance — when it comes to microbial dynamics, the surrounding biotic and abiotic neighborhoods play important and potentially inseparable roles. It also raises a number of interesting questions for future work. Although the authors used 16S rRNA metagenomic analysis to track large-scale compositional changes in their microbial communities, these changes were not correlated in an obvious way with the community-driven changes in focal strain fitness and selection (though the changes did differ systematically between the gentamycin and kanamycin experiments). Future work may aim to link the mesoscale ecological mechanisms described in the new work [3] with detailed molecular mechanisms — perhaps, for example, tying the kanamycin protective effect to the presence of specific drug-modifying enzymes. In addition, it would be interesting to investigate community effects in other complex environments where selection may be driven by horizontal gene transfer, spatial heterogeneity — including that imposed in biofilm communities — or environmental fluctuations not present in the current experimental setup.

From a theoretical perspective, the approach taken by Klumper *et al*. [3] — in which the effects of a complex community are reduced to coarse-grained competition coefficients — bears at least superficial similarity with classical methods in statistical physics, where interactions with 'the environment' — essentially, everything other than the specific system under study — are replaced by coupling to a 'heat bath', an abstract external perturbation that swaps intractable microscopic dynamics — for example, the kicks and whims of every single molecule — for a fluctuating black box, a statistical encapsulation of macroscopic trends arising in large ensembles. Since the pioneering work of May [16], similar concepts have become core tenants of modern ecological theory, indicating that shared statistical principles — manifested as random interaction matrices in large communities [17], symmetry breaking that produces particular compositional states [18], distinct networks governing species interaction and replacement [19], or cyclic interaction patterns that promote biodiversity [20], just to name a few — may unify the large-scale behavior of seemingly disparate ecological systems. An increased understanding of these dynamics, both experimental and theoretical, will be essential for controlling and predicting the dynamics of the complex microbial communities increasingly linked with human health. And in the long run, a deeper understanding of microbial societies — and the interplay between the ecological, statistical, and molecular forces shaping their collective phenotypic wizardry — may inspire evolutionarily sound strategies for prolonging our antibiotic era.

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