Antibiotics: Conflict and Communication in Microbial Communities

Antibiotics mediate species interactions in natural habitats, affecting the dynamics of microbial coevolution

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The discovery that microbes produce antibiotic compounds that kill or inhibit other microbes revolutionized modern medicine by offering a means to treat microbial infections. Thus, the “golden age” of antibiotic discovery began when hundreds of antibiotics from diverse microbial genera were characterized for their efficacy in controlling pathogens and the infections they cause. When used at subinhibitory concentrations, however, antibiotics can alter microbial gene expression, suggesting that they also act as signaling compounds.

The threat of a postantibiotic era in human medicine is galvanizing another search for novel antibiotics, raising questions about what ecological conditions are likely to lead to novel or inhibitory phenotypes. Meanwhile, some experts are also seeking to identify environmental reservoirs of antibiotic resistance genes, while wondering what maintains antibiotic resistance in natural habitats. Similarly, agricultural scientists, who are interested in managing soil microbes as an alternative means to inhibit plant pathogens and reduce reliance on pesticides, do not know what selects for broadly antagonistic communities of soil microorganisms.

Recent research in our lab and by others is shedding light on how antibiotics mediate species interactions in natural habitats and on how they affect the dynamics of microbial coevolution. Insights into the specific roles of antibiotics in naturally occurring communities can not only lead us to antibiotic discoveries, but may help explain how distinct phenotypic structures are generated and how to better manage microbial communities for specific functions.

Antibiotics as Weapons among Microorganisms

Antibiotics act as weapons in mediating microbial species interactions in a variety of habitats. For example, antibiotic-producing Pseudomonas, Streptomyces, Bacillus, and Trichoderma spp. are abundant in plant disease-suppressive soils, and these microorganisms are used widely as inoculants against bacterial, fungal, and oomycete plant pathogens (Fig. 1). Similarly, fungus-farming ants form symbioses with antibiotic-producing bacteria in the genus Pseudonocardia to protect their gardens against infections. Such associations with microorganisms appear to be common among insects and marine sponges, reinforcing the idea that antibiotics act as weapons in natural communities.

In these mutualisms, the immediate fitness...
benefits of antibiotics accrue not to the microbe, but to a third party—either a symbiotic plant, ant, or sponge—consistent with complex ecological and evolutionary dynamics. While some studies document benefits of antibiotic production to bacterial fitness in complex microbial communities, they say little as to how antibiotics enhance fitness.

We reasoned that, if antibiotic weapons confer any direct benefits to the producer, then selection should favor antibiotics that are most active against sympatric competitors—that is, similar species from nearby habitats. Focusing specifically on *Streptomyces*, gram-positive bacteria that are prolific producers of antibiotics, we tested whether antibiotic inhibition is greater among microbes from the same (sympatric) or different (allopatric) locations in soil. We find that sympatric *Streptomyces* isolates are more inhibitory against one another than are allopatric isolates,
suggesting that antibiotics serve as weapons that confer fitness benefits to bacteria that produce them.

Further evidence that antibiotics mediate interactions between competitive species comes from studying differences between resource use and inhibition among sympatric and allopatric *Streptomyces* populations. Antibiotic weapons are broadly assumed to mediate competition for resources. If so, antibiotics should target resource competitors, and selection should favor antibiotics that are most inhibitory towards the strongest competitors for resources.

Among a collection of 69 *Streptomyces* isolates, we determined the niche overlap, or similarity in resource use preferences, among pairwise isolate combinations when they were grown on 95 carbon sources to measure resource competition. Among sympatric but not allopatric *Streptomyces* isolates, those that had greater niche overlap inhibit one another more than isolates with less niche overlap. Thus, within sympatric, coevolved microbial populations, antibiotics inhibit the strongest resource competitors, affirming a specific role for antibiotics in mediating microbial resource competition to enhance fitness of antibiotic producers.

**Antibiotics Acting as Signals among Microorganisms**

Amid substantial evidence for antibiotics being weapons, recent research also makes a compelling case for antibiotics acting as signals. At subinhibitory concentrations, antibiotics modulate a wide variety of functions, including stress response, metabolism, biofilm formation, and virulence among diverse bacterial taxa. These and other examples led to the hypothesis that antibiotics in nature act predominantly as signals, mediating species interactions.

However, studying antibiotics in natural habitats is difficult because of inherent challenges in manipulating their concentrations in situ. To address such questions, we studied the effects of subinhibitory antibiotics on competitive interactions among diverse *Streptomyces* isolates in vitro. Specifically, we analyzed the effects of subinhibitory concentrations of five different antibiotics on nutrient use and resource competition for 72 *Streptomyces* isolate pairs.

Although responses vary from one antibiotic to another, exposing some *Streptomyces* to subinhibitory concentrations of antibiotics leads to significant shifts in resource use. In some cases, we saw substantially reduced resource competition for 20–40% of isolate combinations, depending upon the specific antibiotic. Thus, antibiotics may mediate competition to increase competitor fitness, while reducing fitness in others. In this way, antibiotic signals alter the outcome of microbial resource competition, with significant implications for the dynamics of selection for inhibitory, resistance, and resource use phenotypes among coexisting populations.

**Antibiotic Mixed Messages: Implications for Microbial Coevolutionary Dynamics**

Because antibiotics can act as both weapons and signals, they can have a substantial impact on the trajectories of coexisting populations in particular soil settings. While antibiotic weapons can provide fitness benefits to producer species, these molecules simultaneously exert strong selection pressures for resistance to develop in susceptible populations.

Resistance to antibiotics is widely distributed in natural habitats and may be readily acquired through horizontal gene transfers or by mutations. When resistance to one antibiotic spreads within a community, selection will favor microbes that can produce rare or novel antibiotics to which there is little resistance in targeted competitors. Reciprocal selection may thus generate a coevolutionary arms race between antibiotic-inhibited and -resistant populations in which interacting populations accumulate ever-greater capacities to produce or resist antibiotics (Fig. 2).

Microbial communities that support intense competition and are coevolutionary hotspots are likely to maintain populations with diverse antibiotic inhibitory and resistance phenotypes. These communities may be especially fruitful targets for the discovery of novel antimicrobial compounds and may also be highly desirable to cultivate within agricultural soils, where an important goal is to inhibit pathogens that infect valuable crops. However, although there is evidence antibiotic inhibitory capacities accumulate in some microbial communities, sustained antibiotic arms races are likely to be constrained by the costs of maintaining complex antibiotic production and resistance capacities.

Niche differentiation offers a distinct coevolutionary pathway within coexisting populations.
that may reduce the costs of inhibitory and resistance phenotypes. Because antibiotic weapons target competitor species with similar resource use preferences, coevolutionary character displacement via niche differentiation may reduce or eliminate antibiotic inhibitory phenotypes. Thus, rather than ever-more-antagonistic antibiotic-producing competitors, selection may favor resource-specialized microbial populations that avoid producing costly antibiotics and minimize resource competition.

These resource specialists are expected to use resources more efficiently and have greater fitness relative to generalist populations. Further, in the absence of resource competition, antibiotics may confer little fitness benefit and thus be lost from populations. The parallel processes of niche differentiation and loss of costly antibiotic-producing phenotypes suggest a coevolutionary trajectory leading to a community dominated by efficient but nonantagonistic resource specialists. These populations likely maintain some level of antibiotic resistance to protect against antibiotic-producing antagonists.

If antibiotic weapons induce either an arms race or niche differentiation, what are the effects of antibiotic signals on microbial coevolution? More specifically, how might such signals influence the accumulation of inhibitory and resistance phenotypes or resource use preferences?

For competing populations in which antibiotic signals reduce competition by altering patterns of resource use, antibiotic signals may reinforce coevolutionary niche differentiation among
competitors. In contrast, antibiotic signals that increase the significance of competition to microbial fitness and that reduce capacity of a competitor to metabolize preferred resources may select for variants of the signal-producer that produce greater quantities of that antibiotic signal (Fig. 2). This outcome could provide an important transition between antibiotic signals that are produced at very low concentrations and antibiotic weapons that are produced at high concentrations. For example, signaling interactions that reduce the competitive fitness of a sympatric population may be a first step in initiating an arms race.

However, in cases where a signal enhances fitness of a competitor at a cost to its producer, variants that no longer produce the antibiotic likely will be selectively enriched, eliminating a role for that antibiotic in coevolutionary interactions. Whether antibiotic signals promote coevolutionary trajectories of niche differentiation versus an arms race will depend on whether signals mediate fitness-enhancing or -reducing interactions between the signal-producer and its targets (Fig. 3). Other factors, including habitat characteristics, frequencies of microbial invasion or habitat disturbance, microbial phylogeny, and habitat stability will also influence coevolutionary trajectories.

**Coevolutionary Outcomes and Fitness Tradeoffs**

Arms races or niche differentiation presumably yields populations of antagonistic competitors or growth-efficient resource specialists, respectively. The role of antibiotics in species interactions differs fundamentally for these two different life histories. Antagonistic competitors rely on antibiotics as weapons, while growth-efficient nutrient specialists use antibiotics as signals, if at all. This picture, however, presumes that populations can be either efficient and niche specialized or highly antagonistic, but not both.

However, tradeoffs between antibiotic production or resistance and other microbial life histories remain relatively unexplored. While fear of highly virulent and simultaneously highly antibiotic-resistant pathogens remains widespread, there is little information on the cumulative costs of resistance or inhibition for microbial populations. Both antibiotic production and resistance can impose fitness costs in the laboratory, but compensatory mutations might reduce or eliminate the costs of antibiotic resistance for microbial pathogens in clinical settings. Moreover, antibiotic biosynthesis is often tightly regulated so that antibiotics are produced only in response to resource limitation, microbial signals, or at particular life stages, which may limit the metabolic costs of antibiotic production to situations where they are needed. Can microbes solve the jack-of-all-trades, master-of-none dilemma through compensatory mutations and tightly regulated gene expression?

Our studies of naturally occurring *Streptomyces* populations shed light on the potential for fitness tradeoffs to limit life history strategies. Specifically, if fitness tradeoffs constrain the capacity for a microbe to grow efficiently while being highly antagonistic, then microbes that produce more antibiotics or that can inhibit a wider range of target populations theoretically will grow less efficiently on average than organisms that are less antagonistic.

Among a random collection of 263 *Streptomyces* from prairie soil, we found that isolates that were highly antagonistic (inhibited all 10 target *Streptomyces*) grew significantly less efficiently on average (22% less growth) than did...
isolates that were unable to inhibit any of 10 target isolates. Thus, in these naturally occurring populations, there is evidence for fitness trade-offs between antibiotic-inhibitory phenotypes and growth efficiency, affirming the likelihood of an either/or coevolutionary trajectory for competing populations.

Antibiotic Interactions within Complex Communities

Microbial responses to antibiotics typically are analyzed within the context of pairwise or perhaps three-way species interactions. However, microbes live in complex communities, harboring an astounding phylogenetic and functional diversity in which multiple antibiotics serve simultaneously as weapons and signals amid intricate webs of interactions (Fig. 4).

In this context, an antibiotic may be mutualistic, synergistic, or manipulative, acting as either a weapon or a signal depending upon the interacting species. Moreover, among coexisting populations, there are likely to be keystone taxa that have disproportionately large fitness impacts and thus play a more central role in determining ecological and coevolutionary dynamics within the community. Studying coevolved populations in natural habitats offers a powerful resource for expanding our understanding of these complex dynamics.

The amazingly diverse antibiotic arsenal is critical not only to the biology of microbes, but to management of human, animal, and plant diseases. A comprehensive understanding of the ecology of antibiotics will help us to develop new means for discovering novel antibiotics while better capitalizing on the diversity of natural products, effectively managing soil communities to suppress plant diseases, and analyzing microbial dynamics among naturally occurring populations.

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Suggested Reading


FIGURE 4

Antibiotic-mediated networks of interactions within natural communities can be complex. In these networks each node represents an individual microbial population, and vectors represent direct interactions between populations. Antibiotic weapons (red lines) or antibiotic signaling interactions (blue lines) will mediate interactions between population pairs, but may also influence populations indirectly. Thus, for example, species B may directly inhibit species C, but species D may indirectly influence fitness of species C by limiting the potential for species D to inhibit species C. Keystone species (e.g., species D) may have especially large direct and/or indirect impacts on species interactions and coevolution within a community.
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