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Using ecological coexistence theory to understand antibiotic resistance and microbial competition

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Tackling antibiotic resistance necessitates deep understanding of how resource competition within and between species modulates the fitness of resistant microbes. Recent advances in ecological coexistence theory offer a powerful framework to probe the mechanisms regulating intra- and interspecific competition, but the significance of this body of theory to the problem of antibiotic resistance has been largely overlooked. In this Perspective, we draw on emerging ecological theory to illustrate how changes in resource niche overlap can be equally important as changes in competitive ability for understanding costs of resistance and the persistence of resistant pathogens in microbial communities. We then show how different temporal patterns of resource and antibiotic supply, alongside trade-offs in competitive ability at high and low resource concentrations, can have diametrically opposing consequences for the coexistence and exclusion of resistant and susceptible strains. These insights highlight numerous opportunities for innovative experimental and theoretical research into the ecological dimensions of antibiotic resistance.

Less than a century since the discovery of antibiotics marked one of medicine's greatest forward leaps, the evolution of resistance has emerged as one of its most pressing challenges[1]. So far, efforts to address this global health problem have primarily concentrated on restricting unnecessary prescriptions, the development of new antibiotics and the use of novel combinations of existing antibiotics[2]. Now, however, with emerging insight into the critical role the host and environmental microbiomes play in regulating health and diseases, there is growing consensus that we need a deeper mechanistic understanding of how competition, both within and between species, modulates antibiotic resistance[3–7]. This expanded ecological perspective holds promise not only for our fundamental understanding of resistance evolution, but also for the identification and development of new treatment strategies.

A core challenge in tackling antibiotic resistance is understanding the balance between the selective advantage conferred by a resistance allele and the selective disadvantage incurred by any associated fitness trade-offs, that is, so-called costs of resistance[8,9]. Simple population models predict that because resistant and sensitive strains compete within the same habitat (for example, within hosts or the environment), the fitter strain—determined by the level of antimicrobial exposure and the costs of resistance—should exclude the weaker strain[10]. In reality, however, an unexpected observation is that sensitive and resistant strains commonly coexist even though they compete within hosts[10–12], and that resistant strains often persist long after antibiotic exposure has ceased[13]. Not knowing how and why resistant and sensitive strains coexist is widely recognized to be a major barrier to predicting the prevalence of resistance[10,14,15], and ultimately prolonging the effective therapeutic life of antimicrobials[13].

By convention, costs of resistance are defined as a decrease in the fitness (for example, per capita growth rate or yield) of a mutant strain possessing a resistant allele relative to an isogenic, ancestral strain in an antibiotic-free environment[8,9]. In practice, costs of resistance are usually evaluated based on comparisons of demographic parameters in monoculture, or preferably based on the relative frequency of the mutant strain and the ancestral strain after 24 hours competing in batch culture[16]. Thus, ecologically speaking, a cost of resistance is analogous to a loss in competitive ability. Competitive ability is of course highly context dependent; a good competitor in one environment may be a poor competitor in another. Indeed, a number of studies have demonstrated the environmental contingencies of resistance costs[17–21]. Perhaps less obvious, and certainly much less studied, is the extent to which competitive ability depends on biotic context beyond the focal bacteria, that is, the identity of community members and the propensity for competitive interactions between them. A large loss in competitive ability may be negligible in a depauperate community free from competitors, whereas conversely a small loss in competitive ability may have important implications for survival in a more healthy, diverse system.

In the ecological literature, a rich body of theory has built up over the past 30–40 years with the primary goal of understanding the dependence of pairwise competitive outcomes on the broader community of interacting species[22–30]. Through a quantitative partitioning of differences in competitive ability and niche overlap between competing species, coexistence theory (as it is popularly termed) has been especially instrumental in crystallizing the fundamental rules of diversity maintenance and providing a mathematical framework to investigate the relative importance of deterministic and stochastic processes for coexistence in empirical systems[23,28,29]. Note that niche overlap is not a measure of cohabitation, but rather the degree to which species overlap in resource use in space and time. For example, two species may cohabit the gut but if they never interact, either directly or indirectly, they are said to have zero niche overlap. In addition, coexistence theory has been invaluable in disentangling contradictory perspectives on competitive ability and coexistence in equilibrium and non-equilibrium systems[31–33]. While this framework has been embraced by community ecologists focusing on interspecific interactions, when reproduction is primarily clonal, as it is in many bacteria, the framework can be applied equally robustly to intraspecific interactions (that is, competition between different resistant and susceptible genotypes within a species).

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Despite the relevance of ecological theory for understanding the evolution of resistance, the separation of these two research topics into largely independent literature has slowed their synthesis. Several research groups have begun to bridge this gap, with a number of studies illustrating the important role within-host ecology can play in regulating the evolution of resistance in complex communities[6,7,15,34]. Nevertheless, advances in our understanding of coexistence at the frontier of the field remain largely untapped. Our primary goal here is therefore to highlight core concepts from coexistence theory that have the potential to inform our understanding of how antibiotic-resistant strains are able to persist alongside susceptible strains and/or the broader microbial community. We begin, in 'Partitioning costs of resistance', by outlining the fundamental tenets of coexistence theory and the insights that can be gained from partitioning costs of resistance into effects on competitive ability and effects on niche overlap, before exploring the existing empirical rationale for the partition. In 'Coexistence and resistance in dynamic environments', we look deeper into the coexistence of susceptible and resistant strains in constant versus variable environments, drawing on emerging ecological theory on the effects of equilibrium versus non-equilibrium dynamics on competitive ability. Under 'Limitations', we identify some of the key drawbacks of existing ecological theory as applied to antibiotic resistance evolution, and how theory might be developed to address these limitations. We finish, in 'Future directions', by identifying what we perceive to be the most important theoretical and empirical gaps in our understanding of the community ecology of costs of resistance.

Partitioning costs of resistance

While coexistence theory embodies a broad body of theory aimed at characterizing the diverse mechanisms regulating coexistence in spatiotemporally homogenous and heterogeneous environments, one of its defining features is to separate the contributions differences in competitive ability and niche overlap make to the relative fitness of a focal species and its competitors[23,25,28]. Note that differences in competitive ability are also commonly referred to as fitness differences, but we adopt the former terminology here to avoid confusion with evolutionary fitness, which is the combined outcome of differences in competitive ability and niche overlap. Competitive ability differences capture how well adapted two species are to their shared environment, whereas niche differences capture how much they overlap in their usage of their shared environment. It follows that coexistence between competitors requires differences in competitive ability not to exceed the stabilizing effect of niche differences. At the extreme, if two species completely overlap in resource usage (and share the same predators) in space and time, then they would need to have identical competitive ability to coexist, and even then only neutrally. However, the more species differentiate in resource usage, the greater the range of differences in competitive ability that are compatible with coexistence, until the other extreme is reached, where two species that have zero niche overlap (that is, do not use any of the same resources in space and time) can coexist in spite of infinitely large differences in competitive ability (see Box 1).

How then might separating relative fitness into competitive ability and niche overlap components inform our understanding of antibiotic resistance evolution? The critical insight is that costs of resistance may arise either solely from a loss in competitive ability, or in conjunction with a change in niche overlap. Traditionally, costs of resistance have been interpreted primarily through the lens of a loss in competitive ability (that is, a reduction in growth rate or yield on a given resource)[16]. This might be reasonable, under certain assumptions, if we are concerned only with the relative fitness of a resistant mutant and its antibiotic-susceptible ancestor, but in the presence of a multispecies community the limitations of ignoring niche overlap come into especially sharp focus.

Box 1 | Separating competitive ability and the niche

Although differences in competitive ability and niche overlap can hypothetically be obtained for any pairwise model of competition, the most convenient formulas, and the most readily used by empirical ecologists, are those derived for phenomenological Lotka–Volterra-type competition models of the general form:

$$\frac{dN_i}{dt} = N_i(r_i - \alpha_{ii}N_i - \alpha_{ij}N_j), \quad (1)$$

where N_i is the abundance of species i, N_j is the abundance of species j, t is time, r_i is the per capita intrinsic rate of increase of the focal species, α_{ii} is the linear effect of intraspecific competition (sometimes parameterized as carrying capacity, $K = \frac{1}{\alpha_{ii}}$) and α_{ij} is the linear effect of interspecific competition of species j on species i.

Niche overlap, ρ, is given by the geometric mean ratio of the intraspecific and interspecific coefficients

$$\rho = \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}}, \quad (2)$$

and the competitive ability ratio, $\frac{k_j}{k_i}$, is given by the ratio of intrinsic growth rates multiplied by the geometric mean ratio of each species' sensitivity to competition

$$\frac{k_j}{k_i} = \frac{r_j}{r_i}\sqrt{\frac{\alpha_{ii}\alpha_{ij}}{\alpha_{jj}\alpha_{ji}}}. \quad (3)$$

Based on the mutual invasibility criterion for coexistence, that is, that each species can invade from rare when its competitor(s) is resident at its equilibrium density, stable coexistence requires that

$$\rho < \frac{k_j}{k_i} < \frac{1}{\rho}. \quad (4)$$

In other words, as niche overlap increases (approaches 1), an increasingly narrow range of differences in competitive ability are compatible with coexistence (see Fig. 1b).

For more mechanistic models of competition (for example, consumer–resource models), which we use here to illustrate how costs of resistance can be partitioned into effects on competitive ability and niche differences, under certain assumptions we can obtain these metrics by obtaining mechanistic definitions for the Lotka–Volterra parameters (see Supplementary Information S1). Competitive ability and niche differences then become explicit functions of the consumer growth and resource supply parameters, rather than the competition coefficients of Lotka–Volterra.

Consider a mutant that evolves resistance to an antibiotic resulting in either: reduced growth on all resources consumed by the ancestor, that is, a general metabolic burden (R_i, bottom left in Fig. 1a); or zero growth on just a subset of resources consumed by the ancestor, that is, more specific changes in bacterial physiology (R_{ii}, bottom right in Fig. 1a). The implications these different scenarios have on partitioning costs of resistance are illustrated in Figs. 1 and 2 via the analysis of a classic mechanistic model of competition for two substitutable resources (see Supplementary Information S1 and S2 for details).

Despite very different trade-offs in their resource utilization traits, both mutant phenotypes exhibit identical costs of resistance in terms of reductions in their Malthusian growth parameters

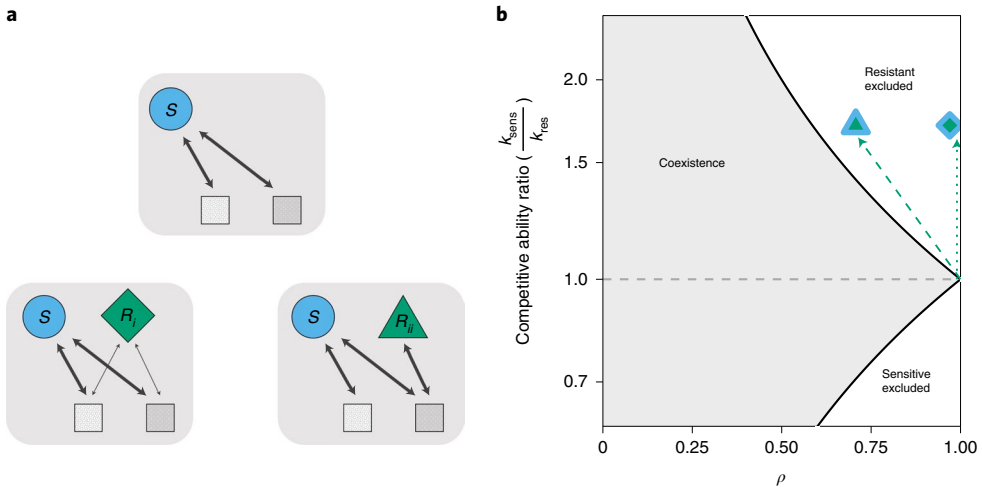

Fig. 1 | Partitioning costs of resistance mediated by constraints on resource uptake into competitive ability differences and niche overlap. a, Basic food webs for an antibiotic-susceptible pathogen (*S*) consuming two substitutable resources in the absence (top) and presence (bottom) of two different antibiotic-resistant mutants, exhibiting either an equal magnitude loss in performance on both resources (R_i, bottom left) or a loss in the ability to metabolize one of two resources with no change in performance on the other resource (R_{ii}, bottom right). Line weight indicates consumer per capita growth rate for each resource, and bidirectional arrows indicate that consumers and resources have reciprocal feedbacks on their respective densities. **b**, Dotted and dashed green arrows trace the corresponding change in niche overlap (ρ) and competitive ability differences between the susceptible and resistant strain (R_i, green-filled blue diamond; R_{ii}, green-filled blue triangle). Grey shaded region indicates parameter space corresponding to coexistence; unshaded region indicates parameter space corresponding to exclusion. See Supplementary Information S2 for model parameters.

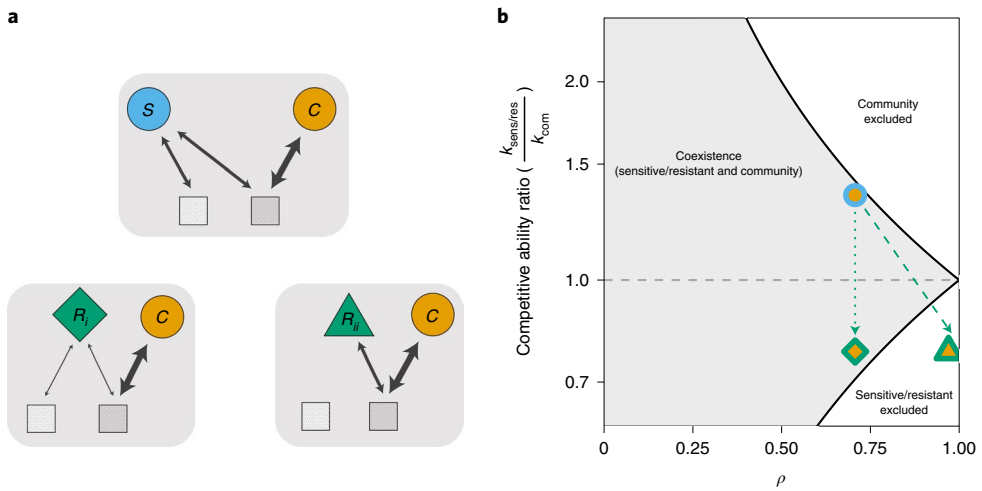

Fig. 2 | Partitioning costs of resistance mediated by constraints on resource uptake into competitive ability differences and niche overlap. a, Basic food webs for a non-pathogenic member of the background community (*C*) consuming one of two substitutable resources in the presence of either an ancestral antibiotic-susceptible strain (top), or two alternative antibiotic-resistant mutants (bottom) exhibiting either an equal magnitude loss in performance on both resources (R_i, bottom left) or a loss in the ability to metabolize one of two resources with no change in performance on the other resource (R_{ii}, bottom right). Line weight indicates consumer per capita growth rate for each resource, and bidirectional arrows indicate that consumers and resources have reciprocal feedbacks on their respective densities. **b**, Orange-filled blue circle denotes the initial niche overlap and competitive ability differences between the susceptible strain and the background community member. Dotted and dashed green arrows trace the corresponding change in niche and competitive ability differences between the resistant strain and the background community member (R_i, orange-filled green diamond; R_{ii}, orange-filled green triangle). See Supplementary Information S2 for model parameters.

(growth rate at low density) and carrying capacities relative to the ancestral strain (see Supplementary Information S2). This is to say that the apparent costs of resistance of the two mutant phenotypes will be indistinguishable based on measurements of their growth in monoculture. From the perspective of coexistence theory, however, these costs of resistance present very different targets for control by commensal members of the background community.

Consider first the relationship between the ancestral strain and the two resistant phenotypes (Fig. 1). Relative to the ancestral strain, a reduced ability to consume all resources (bottom left in Fig. 1a and dotted green line in Fig. 1b) will result in a reduction in competitive ability but no change in niche overlap. In contrast, losing the capacity to metabolize a previously consumed resource (bottom right in Fig. 1a and dashed green line in Fig. 1b) will not only result in the

same reduction in competitive ability but also a reduction in niche overlap relative to the ancestral strain. In both cases, however, the outcome will be exclusion of the mutant strain by the susceptible in the antibiotic-free environment. It is nevertheless feasible that the mutant strain could gain access to (or better utilize) a resource that the ancestral strain does not utilize (or utilizes poorly), in which case the resultant change in niche overlap could be sufficient to foster the instantaneous coexistence of the resistant and susceptible phenotypes (see 'Empirical basis').

Now consider the relationship between the mutant phenotypes and a member of the background community. The significance of an increase in niche overlap between an antibiotic-resistant mutant and the background community depends to some extent on environmental context. In the absence of antibiotics, an increase in niche overlap between the antibiotic-resistant mutant and the background community could bring them into more direct competition, and therefore exaggerate observed costs of resistance (Fig. 2b). This could be particularly important in preventing the evolution of resistance if the loss of competitive ability between the mutant strain and the ancestral strain is comparatively small. In contrast, in the presence of an antibiotic, any change in niche overlap between the mutant strain and the background community will be of little consequence if the members of the background community are also inhibited by the antibiotic. However, if some background community members are naturally insensitive to the antibiotic (for example, in the case of narrow spectrum antibiotics that target only gram negative or positive bacteria), they could play a critical role in preventing an antibiotic-resistant mutant from dominating the system[7,15,34].

Similarly, compensatory mutations that reduce the apparent costs of resistance can be usefully partitioned into their effects on competitive ability and their effects on niche overlap. For example, a compensatory mutation that reverses a resistance-associated decline in resource efficiency (that is, a general metabolic burden) should equalize the competitive ability of the resistant and susceptible strain, and bring them closer to a state of potential neutral coexistence (a reversal of the green dotted arrow in Fig. 1b). The same would be true if a compensatory mutation restored the ancestral ability to metabolize a particular resource (a reversal of the green dashed arrow in 1b). It is also possible, however, for a compensatory mutation to stabilize coexistence more strongly with its antibiotic sensitive ancestor in spite of no change in the competitive ability of the resistant strain. This would arise if the resistant strain was to acquire access to a resource that the ancestral susceptible strain is unable to use.

Empirical basis. There is substantial evidence that co-occurring microbes can vary substantially in their competitive ability for different nutrient niches[35], with communities associated with both animal hosts and soils exhibiting particularly high levels of metabolic differentiation[36]. At the same time, there is growing awareness that resource availability and dynamics can play an important bottom-up role in regulating pathogen dynamics[37–40]. To our knowledge, however, no studies to date have looked directly at partitioning apart changes in niche overlap and competitive ability arising from resistance mutations. Nevertheless, there is notable evidence that resistance mutations are frequently associated with specific metabolic shifts rather than a more general metabolic burden[19,41–45].

For example, an experimental study[42] found that the uptake of several carbon substrates utilized strongly by rifampicin-susceptible strains of *Bacillus subtilis* was no longer detectable in many resistant strains. This would be consistent with both a change in the competitive ability ratio and niche overlap between the wild type and resistant mutants, as illustrated by the green-filled blue triangle in Fig. 1b. The authors also found, however, that many mutant strains increased their utilization patterns on substrates utilized only weakly by the wild type[42]. The implication is that a single resistance mutation could actually result in a sufficiently large decrease in niche overlap to allow the susceptible ancestor and the resistant mutant to coexist, that is, the green-filled blue triangle in Fig. 1b would fall within the shaded coexistence region. Similarly, another study[19] found that streptomycin-resistant *Salmonella typhimurium* were better able to utilize several (poorer) carbon sources than the wild type. Despite apparent fitness costs arising from reduced growth on favourable carbon sources, this shift in resource utilization patterns may again be sufficient to allow streptomycin-resistant mutants to coexist alongside susceptible strains (assuming the availability of multiple carbon sources). A more recent study[45] found that piperacillin-resistant lineages of *Pseudomonas aeruginosa*, which had lost the ability to catabolize several carbon sources, exhibited enhanced growth on glucosamine. This example is, again, suggestive of the potential for stable (frequency-dependent) coexistence of resistants and sensitives.

The previous examples suggest that chromosomal mutations that have pleiotropic effects on multiple traits are likely to result in both changes in competitive ability and niche overlap between wild type and resistant strains. Are there scenarios under which we might expect niche overlap to remain unchanged in spite of changes in competitive ability? One pathway by which fitness costs may exclusively affect competitive ability is when resistance is conferred through the overexpression of multidrug efflux pumps that result in resources being ejected before they can be used[46,47]. That being said, the overexpression of a multidrug efflux pump in *Stenotrophomonas maltophilia* has been shown to make resistant mutants more efficient in acquiring certain sugars and amino acids than the wild type[41].

An alternative route to competitive-ability-centric fitness costs is plasmid carriage. The main fitness costs of plasmid carriage are thought to stem from the translation of protein-encoding plasmid genes, which diverts limiting resources and energy, including adenosine triphosphate, from other critical processes within the cell. This increased metabolic burden has been associated with reduced growth rates and cell densities, and lengthening of lag phases[48,49]. To the extent that these increased energetic costs will have broad cell-wide effects on metabolic fluxes and cellular processes[48], it may be expected that plasmid carriage will have minimal impacts on niche overlap despite notable fitness costs. Nevertheless, plasmid carriage can also be associated with very specific phenotypic changes, for example via catabolic gene clusters that endow recipient cells with the ability to degrade new chemical compounds[50], or by modifying the expression of chromosomal metabolic genes[48,51]. Examples of the latter include increased expression of genes encoding glutamine synthesis[52] and iron acquisition[51] in *Pseudomonas* spp., both of which should have notable effects on both competitive ability and niche overlap. It is plausible that carriage of catabolic plasmids encoding novel metabolic enzymes could even facilitate coexistence of non-plasmid- and plasmid-carrying strains; however, so far there appears to be little evidence of plasmids carrying both degradative and resistance genes[53,54].

Together with the preceding hypothetical scenarios, these examples illustrate the potential insights to be gained through the partitioning of costs of resistance into changes in competitive ability and niche overlap. However, we have so far only considered competition in an equilibrium system, and ignored the influence of temporal variability. From ecological theory[23,28,30] we know that temporal variability can play an important role in maintaining coexistence when competing species trade-off along a time-varying niche axis (for example, antibiotic or nutrient concentrations). In 'Coexistence and resistance in dynamic environments', we turn our attention to the role of constant (equilibrium) versus fluctuating (non-equilibrium) patterns of antibiotics exposure and resource availability on competitive ability and costs of resistance. Whereas in the preceding

section we considered coexistence at both the intraspecific (between susceptible and resistant strains) and interspecific level (between resistant strains and commensal members of the microbiome), to explore the effects of fluctuating conditions we now focus on competition between resistant and susceptible genotypes. Nevertheless, the underlying theory, predictions and implications apply equally to understanding competition between resistant pathogens and other heterospecific members of the community.

Coexistence and resistance in dynamic environments

Microbial communities generally occupy dynamic environments[55], where fluctuations in environmental factors (such as pH, temperature and of course antimicrobials) and nutrient resources can drive substantial oscillations in population abundances and community composition through time. The pulsed nature of most antibiotic dosing regimes probably acts as a particularly potent driver of variability. Nevertheless, despite a substantial body of literature focusing on the optimization of dosing regimes (that is, dose timing and frequency) in the context of pharmacodynamics (for example, ref. [56]), the implications of different temporal patterns of antibiotic delivery on the interaction between within-host competition and the evolution of antibiotic resistance have thus far gone largely ignored. Notwithstanding several recent studies that have begun to tackle this question (for example, refs. [57–61]), and ongoing discussion over the merits of low-dose regimens[62–64], as noted elsewhere[13], "the absence of basic knowledge about ideal prescribing regimens represents a significant gap".

Part of the difficulty in tackling this question is that, in the community context, the efficacy of different dosing regimes should interact closely with the temporal dynamics of limiting nutrients. To our knowledge, there has been little research looking explicitly at how the interaction between resource delivery regime (for example, feeding patterns) and antibiotic exposure regulates the evolution of antibiotic resistance. We can nevertheless draw some insight from emerging research into the effects of feeding patterns, independent of nutritional composition, on gut microbiome stability[65–67].

Even without exposure to antibiotics, the gut is already a highly dynamic environment[35], where natural feeding patterns generate fluctuations in nutrients, pH and secondary metabolites[65]. A wealth of research has already documented the conspicuous effects diet has on the gut microbiota; a change in diet can dramatically shift an individual's gut microbial community in as little as a few hours[68–70]. Much less attention has been given to understanding how patterns of feeding (for example, time restricted versus unrestricted), independent of dietary composition or calorific intake, modulate gut microbiome composition. Nevertheless, a small number of recent studies have provided compelling evidence that feeding patterns can indeed reshuffle competitive hierarchies within the gut[65–67,71,72]. For example, one[65] found that mice subjected to the same diet but different feeding patterns differed in their gut microbiota, with natural cycles of feasting and fasting corresponding to predictable changes in microbial composition. Another[66] similarly found intra-daily feeding patterns to be a strong determinant of microbial cycling within the gut, with mice fed exclusively at night or day exhibiting a corresponding phase shift in the peak abundance of particular taxa.

If, as several recent studies have suggested[6,7,34,63], ecological competition can place strong limits on the evolution of antibiotic resistance, it stands to reason that the strength of this inhibitory force will be highly contingent on competitor identity. At the same time, the aforementioned research suggests that subtle differences in feeding pattern alone can have substantial impacts on the identity of dominant competitors on timescales that closely correspond to the pharmacological lifespan of antimicrobial drugs (that is, hours). In 'Predicting competitive outcomes', we draw on ecological theory to lay out a series of testable predictions for how antibiotic delivery and its interactions with resource availability and resistance trade-offs may be expected to modulate the relative fitness, coexistence and exclusion of resistant and susceptible strains.

Predicting competitive outcomes. All else being equal, under constant antibiotic exposure, a resistant strain will exclude a susceptible strain above some critical threshold in antibiotic concentration, and a susceptible strain will exclude a resistant strain below that threshold. The threshold is set by the point at which the fitness costs of resistance and susceptibility cross over. Most pertinent to the current discussion, however, is that coexistence under this simplistic scenario is not possible, beyond the unlikely case of exactly equal fitness. By contrast, the pulsed delivery (typical of most regimes) of a growth-inhibiting antibiotic can promote stable coexistence of resistant and susceptible strains at the same time-averaged concentration that would lead to one phenotype being excluded under constant exposure (Fig. 3). This occurs because the pulsing of an antibiotic that impedes growth effectively concentrates intra-strain competition relative to interstrain competition in time. In the language of coexistence theory, pulsing decreases niche overlap via a mechanism community ecologists refer to as a temporal storage effect—a formalization of the concept of temporal niche partitioning, where competitors profit under different conditions (for example, presence/absence of antibiotics) in a fluctuating environment[73,74].

Fluctuations in bactericidal antibiotics that kill microbes rather than inhibit growth (bacteriostatic) could also give rise to a temporal storage effect provided additional assumptions are met (for example, negative resource dependence in per capita mortality arising from a starvation survival response[75]). It is instructive to note, however, that irrespective of the class of antibiotic, we would not predict coexistence when the resistant is already favoured under constant antibiotic supply. This is because the resistant strain's insensitivity to antibiotic fluctuations precludes the emergence of a temporal storage effect. As such, this phenomenon has particular relevance to current discussions over the merits of low-dose regimes[62–64], where the temporal pattern of dosing (constant versus pulsed) could be the difference between the suppression or emergence of resistance.

To illustrate this effect of low-dose antibiotic pulsing on coexistence more completely, Fig. 4 summarizes simulations of resource competition between a susceptible strain and a resistant strain characterized by varying degrees of fitness costs (reduction in per capita growth rate) across increasingly large antibiotic pulsing intervals (see Supplementary Information S3 for simulation parameters). The pulsing interval represents switching time between antibiotic-free and antibiotic-exposed conditions. As such, the total (or average) amount of time the competing strains are exposed to an antibiotic is constant across pulsing intervals, but the more frequent the switching, the more constant the bacteria perceive the environment. As in Fig. 3, the costs of resistance are chosen such that when conditions switch rapidly back and forth (bottom row), the susceptible excludes the resistant for all but the smallest costs of resistance. As such, the x axis (resistant growth rate) may be alternatively interpreted as the antibiotic concentration; smaller costs of resistance correspond to an increase in the time-averaged relative competitive ability of the resistant strain (as expected under increasing antibiotic concentrations).

The main observation we wish to highlight is that as the length of the pulse interval increases, the range of fitness costs that enable the resistant strain to persist broadens. This is because the longer the antibiotic pulsing interval, the less the temporal niche overlap between competitors favoured under opposing conditions, and therefore the stronger the temporal storage effect. At the same time, when resistance costs are small (three rightmost columns of Fig. 4), or alternatively antibiotic concentrations are high, no amount of antibiotic pulsing will promote coexistence. In sum, all else being

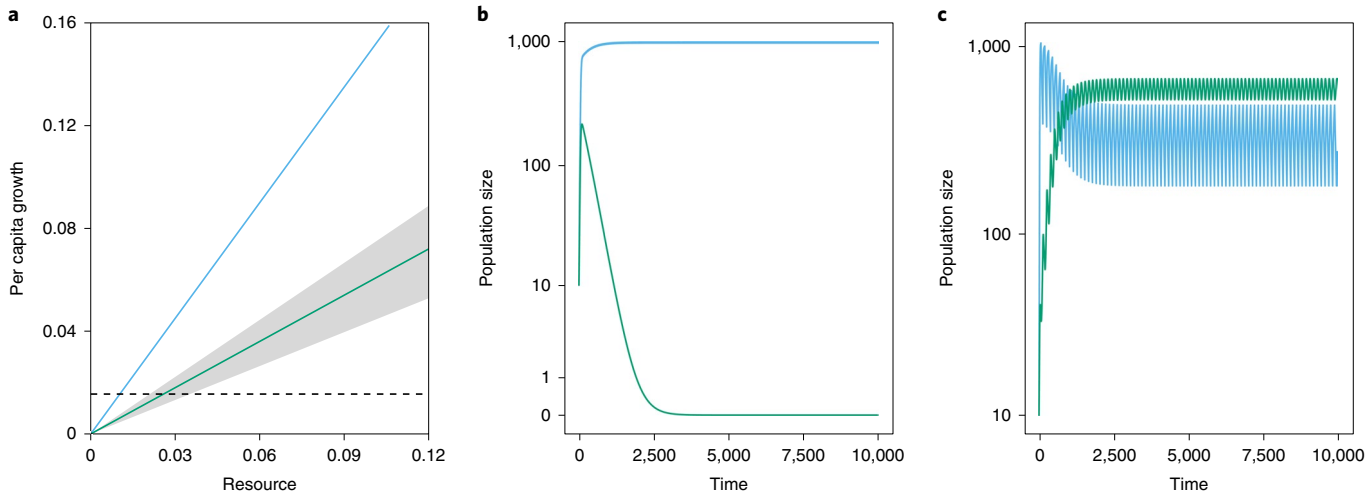

Fig. 3 | The effect of constant versus pulsed delivery of a growth-inhibiting antibiotic on coexistence between a susceptible strain (blue) and a resistant mutant (green). a, Per capita growth responses to a limiting resource under antibiotic-free conditions, with the susceptible strain exhibiting superior growth. Under antibiotic exposure, the susceptible strain is unable to grow (not shown), whereas the resistant strain is unaffected. **b**, When antibiotics are delivered at a constant rate that is too low to favour the resistant strains (that is, the cost of resistance is still too high), the susceptible strain excludes the resistant strain. **c**, Pulsing of antibiotics at the same time-averaged concentration as in **b** allows the resistant strain to coexist alongside the susceptible strain. For the given pulse interval, stable coexistence emerges for all costs of resistance spanning the grey shaded area in **a**. The horizontal dashed line in **a** denotes the density independent mortality rate. See Supplementary Information S3 for model simulation design and parameters.

equal, fluctuating conditions generated by the pulsing of antibiotics favours resistant strains.

But what if all else isn't equal? An assumption of the above simulations was that the limiting resource entered the system at a constant rate, as it would in a chemostat. This of course is unlikely in many microbial environments, including, for example, the animal gut where feeding patterns can cause rapid temporal (hourly to daily) fluctuations in the composition of the microbiota[35,65–67]. Now we relax this assumption to investigate how fluctuations in resource delivery affect the competitive dominance and coexistence of resistant and susceptible strains.

When resources are delivered constantly, and multiple strains (or species) are limited by the same resource, the strain with the lowest maintenance requirement (R^*, resource concentration at which growth rate is zero) will exclude all other competitors[22,24,27,37]. However, when resources fluctuate through time, it is no longer possible for the strain with the lower R^* to maintain the resource at a low level, and so the competitive advantage of being a resource 'gleaner' is dramatically weakened[76]. Instead, an 'opportunist' strain with a higher R^* but a larger growth rate at high resource levels is more likely to be the superior competitor. Indeed, under the right frequency and amplitude of resource pulses, these two strategies can coexist via a mechanism termed relative nonlinearity of competition[74,76,77]. The name of this mechanism refers to the requisite difference in the curvature of strains' growth responses to resource concentration, which allows for the opportunist–gleaner trade-off.

The implication of these opposing resource acquisition strategies is that a cost of resistance that is severe in an equilibrium system may be negligible in a much more variable system and vice versa. Consider an environment in which antibiotics are again pulsed into the system on some periodic schedule (such as the oral ingestion of antibiotics in medical treatment or run-off from agricultural application of antibiotics to livestock). Irrespective of where the resistant strain sits along the resource trade-off spectrum, first note that resource pulses that come out of phase with antibiotics should benefit the susceptible strain, while resource pulses that come in phase with the antibiotics will benefit the resistant strain. This is because an abundance of resources when a strain is favoured by the antibiotic dosing regime will boost that strain's time-averaged competitive ability.

But what if resource availability is not correlated with antibiotic pulsing? This may be the case whenever there are relatively long intervals between antibiotic pulsing (for example, in agricultural settings) or when resource supply (for example, through host consumption) is more frequent than antibiotic dosing. As illustrated via the following simulations, when resources are equally available in the presence and absence of antibiotics, an interaction between the resource acquisition phenotype of the resistant strain and the mode of resource delivery (that is, pulsed versus constant) can have diametrically opposing effects on the outcome of competition.

As in the previous simulations, we make the simplifying assumption that the pulsing interval is regulated by the switching time between antibiotic-free and antibiotic-exposed conditions, such that competing phenotypes experience equal fixed-length periods in antibiotic-free and -exposed conditions. Now, however, we explore the consequences of varying only the resource pulse interval and size (y- and x axes, respectively, in heatmaps in Fig. 5). All resource pulses are nested (that is, shorter in length) within antibiotic pulses. The top rows of the two heatmaps in Fig. 5 represent the case of a single resource pulse coinciding with each switch between the antibiotic-exposed and -free conditions; the bottom row represents the case where >100 resource pulses are provided between each switch in condition. As such, the total amount of resources available is spread equally across the two opposing antibiotic conditions (a dynamic that might reasonably arise under commonly administered 24-hour antibiotic dosing intervals[78]).

First, consider an antibiotic-resistant strain that exhibits the strongest costs of resistance at high nutrient concentrations, that is, the difference in its growth rate relative to the susceptible ancestor is greatest at high resource levels (maximum growth trade-off in Fig. 5; left-hand heatmap). In this case we see that at one extreme, small and frequent resource pulses allow the resistant strain to exclude the susceptible strain (green shaded cells in bottom left of main left panel in Fig. 5). This is because small frequent pulses minimize the fitness difference between the two phenotypes in the antibiotic-free environment. However, with increasingly longer

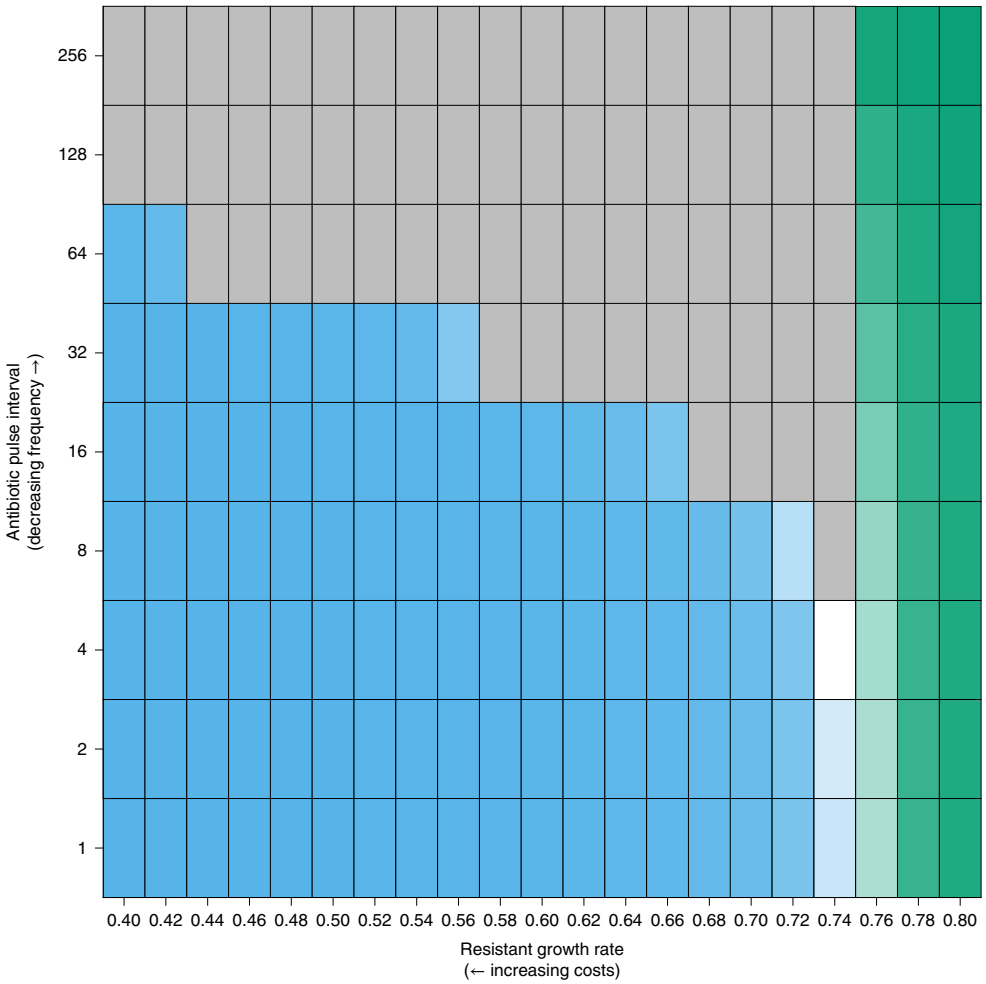

Fig. 4 | Coexistence and exclusion of a susceptible and resistant strain across antibiotic pulse intervals of increasing length but the same time-averaged concentration, and decreasing costs of resistance. In the bottom left corner, large costs of resistance coupled with rapid pulsing results in exclusion of the resistant strain (blue cells). On the right side, small costs of resistance and long pulse intervals result in exclusion of the susceptible strain (green cells). The range of costs of resistance leading to coexistence (grey cells) increases with longer pulse intervals. Lighter cell shading indicates slower rates of competitive exclusion. See Supplementary Information S3 for simulation parameters.

intervals (increase on the *y* axis) and/or larger resource pulses (increase on the *x* axis), the susceptible strain will be able to coexist with the resistant strain (grey shaded cells in the middle of main left panel), until the other extreme is reached and the susceptible strain excludes the resistant strain (blue shaded cells in top right of main left panel). This is because large infrequent pulses maximize the fitness difference between the two phenotypes in the antibiotic-free environment. This prediction is in fact consistent with experimental data obtained in a previous study[79], which found that substantial fitness differences observed between rifampicin-resistant *Escherichia coli* and the ancestral wild type under nutrient-rich conditions largely disappeared when the same strains were competed under nutrient-limiting conditions in a chemostat.

Now consider the alternative scenario, where an antibiotic-resistant strain exhibits the strongest costs of resistance at low concentrations of a limiting resource, that is, the difference in its growth rate relative to the antibiotic-susceptible ancestor is greatest at low resource levels (R^* trade-off in Fig. 5). This is consistent with several experimental studies documenting greater costs of resistance when nutrients are at low concentration[34,80,81]. With an R^* trade-off we see the opposite relationship between resource pulse interval/size and community dynamics. In direct contrast with a maximum growth trade-off, small frequent resource pulses allow the susceptible strain to exclude the resistant strain; intermediate pulse intervals/sizes lead to coexistence; while large infrequent pulses, which minimize costs of resistance, help the resistant strain to exclude the susceptible strain.

To reiterate our main message, in addition to enabling the coexistence of resistant and susceptible strains, a fluctuating resource environment can effectively dial up or down the competitive disadvantage of resistant strains. Whether nutrient fluctuations increase or decrease the competitive ability of resistant strains critically depends on whether fitness costs are smallest at high or low nutrient concentrations, respectively. As such, a simple trade-off in competitive ability for nutrients can reverse the course of selection for antibiotic resistance under opposing patterns of nutrient availability. Although framed here in terms of competition between resistant and sensitive genotypes, these trade-offs should also be expected to modulate the potential for resistant strains to persist alongside the wider microbial community. This is to say, we predict that community-mediated suppression of resistance will also be sensitive to varying temporal patterns in nutrient availability.

Limitations

A possible limitation of much ecological theory in a more clinical context is that the clinical response of interest is often abundance

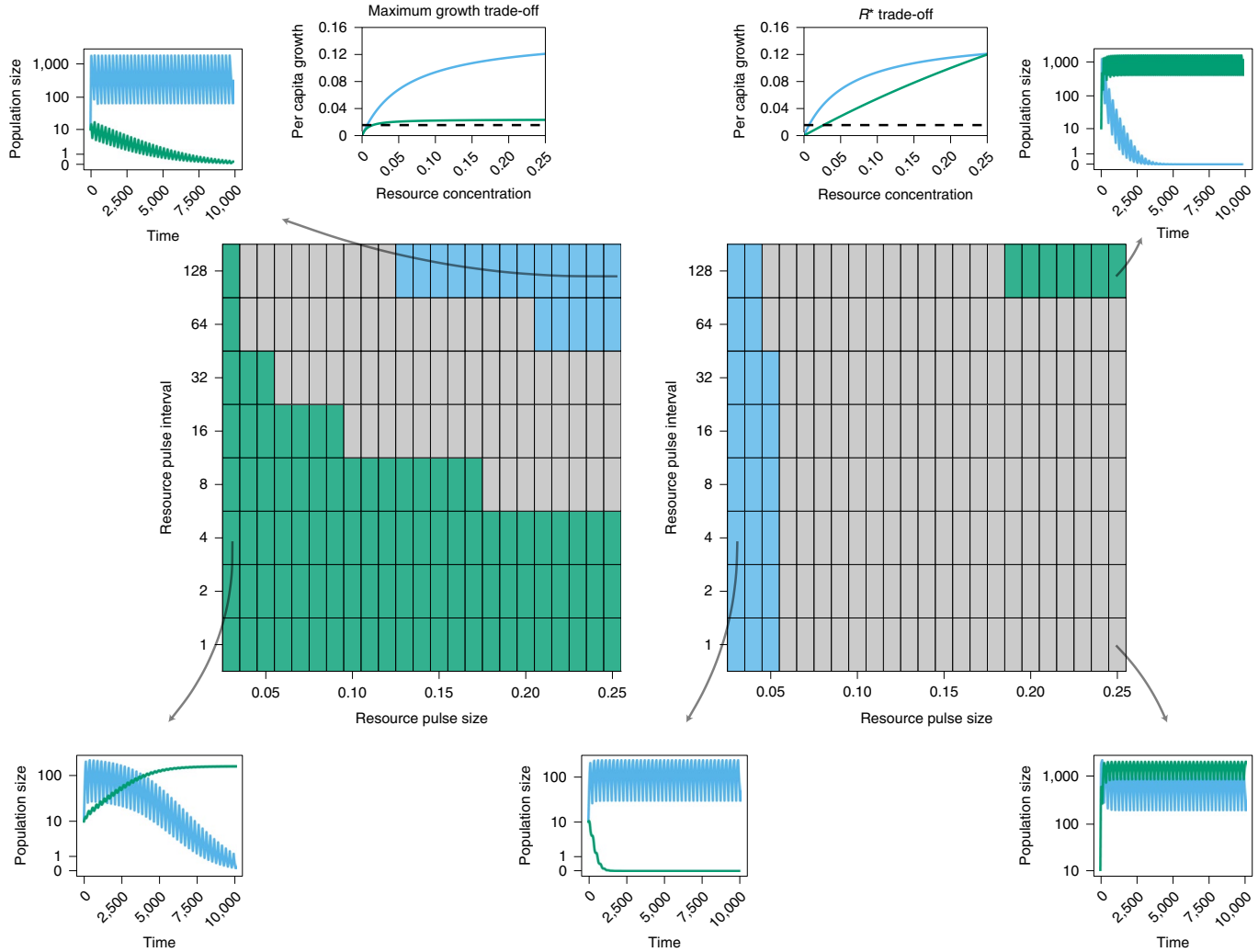

Fig. 5 | Simulation results illustrating the interactive effects of antibiotic pulsing, resource pulse interval length and resource pulse size on competitive outcomes under different resource-uptake-associated costs of resistance (maximum growth trade-off in left panel; *R trade-off in right panel).** The length of each antibiotic-free and antibiotic-exposed interval is fixed at 128 units of time. Total resource availability is equal across the antibiotic-free and antibiotic-exposed intervals. Green cells indicate exclusion of the susceptible strain; blue cells indicate exclusion of the resistant strain; grey cells indicate coexistence. The horizontal dashed lines in the per capita growth response plots denote the density independent mortality rate. See Supplementary Information S4 for simulation parameters.

(that is, pathogen load) rather than qualitative state (that is, coexistence versus exclusion). Ecological theory tends to place more importance on evaluating whether two species can stably coexist than it does on quantifying their relative abundance. However, a pathogen strain that persists at low abundance may be of negligible concern to one that persists at high abundance, even though the systems are qualitatively equivalent. Populations at low abundance also become increasingly vulnerable to demographic stochasticity, and several researchers have argued that the immune system can clear the residual infection once the population is sufficiently small[59]. Fortunately, efforts are already underway to incorporate demographic stochasticity more explicitly into coexistence analysis[82,83], and there is no constraint on extending the types of model regularly adopted by community ecologists to incorporate the immune response.

The latter concern alludes to a much broader tension between theory and reality—that is, the trade-off between generality and complexity. In addition to the immune system, we can make a long list of other ecological and evolutionary factors that may well have an overriding impact on the dynamics identified herein, from cross-feeding, predation, spatial heterogeneity and direct competition (that is, bacterial warfare), to compensatory mutations and horizontal gene transfer. Again there is no limitation to the incorporation of these complexities into the kinds of model we have used for our analyses, and we certainly encourage studying dynamics under these more complex scenarios (see 'Future directions'). Nevertheless, from an ecological perspective, resource competition arguably is the most pervasive and canonical interaction type, at least within trophic levels. Furthermore, owing to energy limitations, anaerobic habitats such as the mammalian gut are thought to support comparatively flat ecosystems[55]. We believe this provides a strong rationale for placing greater research emphasis on the complex role of resource competition in regulating the emergence of resistance.

Finally, whether resistant and sensitive pathogens coexist within individual hosts over the long term remains an open question. There is overwhelming evidence for sustained coexistence at the level of the host population (for example, among patients)[84], and there is increasing evidence of simultaneous carriage of resistant and sensitive genotypes within individuals[85–89]. The time dependence and

scale of this co-occurrence is a concern for both individual and public health because the persistence of resistant genotypes can lead to treatment failure, including increased mortality[4]. It may be that observations of within-host coexistence are typically a transitory phenomenon, and that competitive exclusion of one or more genotypes is the eventual outcome. Nevertheless, even in the absence of long-term stable coexistence, ecological coexistence theory provides a powerful framework for understanding and predicting rates of exclusion and the potential for lengthy transitory states.

Future directions

Experimental tests of the ideas presented represent a natural jumping-off point for future research. The nature and scope of these experiments can be usefully separated into those that explore the partitioning of costs of resistance into niche overlap and competitive ability differences, and those that explore the relative timing of antibiotic and resource pulses on the persistence of antibiotic resistance.

A central question deriving from these analyses is to what extent costs of resistance are disproportionately captured by changes in niche overlap versus changes in competitive ability, and to what extent this balance is contingent on the focal taxa, the type of antibiotic and/or the nature of the resistance mutation. A relatively accessible approach to the partition is to empirically parameterize a competition model (such as Lotka–Volterra), from which niche overlap and competitive ability differences can be quantified (see Box 1)[27,90]. For high-resolution time-series data, the Lotka–Volterra model can be parameterized through statistical fits of the dynamical model (a system of ordinary differential equations)[91]. An alternative approach, common in the plant ecology literature, is to obtain the competition coefficients directly based on statistical fits of per capita growth rate at varying densities of the focal and non-focal strain[92–94].

As previously recognized[13], it remains surprisingly unclear how the timing and frequency of antibiotic dosing affects the evolution of antibiotic resistance. Testing the relevant theoretical predictions presented here would require an experimental setup in which the delivery of antibiotics and resources can be regulated through time from being continuous at one end of the spectrum to highly pulsed at the other. For in vitro work, this almost certainly necessitates a chemostat setup (see, for example, ref. [95]), although a semi-continuous serial transfer approach may be be sufficient under high transfer rates as facilitated by liquid handling robotics. Another approach that would remove the necessity of different antibiotic dosing regimes would be to use antibiotics that vary in their half-life. Looking further forward, there are also opportunities to test these ideas in vivo using model systems such as mice or *Drosophila*, where antibiotic delivery and food availability can be tightly regulated[96].

The experimental approaches outlined in the preceding paragraph all undoubtedly include substantial infrastructural overheads. Building on existing evidence for both R^*[34,80,81] and maximum growth[79] trade-offs arising from resistance mutations, an alternative approach with a lower infrastructural barrier to entry would be to start by simply quantifying the functional nature of resource trade-offs associated with costs of resistance. Which is more common, a loss of competitive ability at high or low resource levels (see, for example, ref. [79])? And does this depend on the limiting resource, the focal taxa, the antibiotic, or the resistance mutation? This information facilitates the parameterization of more mechanistic models of resource competition, which can be used explore the effects of different prescribing regimes on resistance evolution.

Beyond empirical work, there is substantial scope for theoretical studies that incorporate additional complexities into these analyses. There already exists a substantial body of ecological theory aimed at incorporating the effects of predation into the partition of niche overlap and competitive ability[90]. This could be particularly relevant to understanding the combined effects of resource competition and parasitism by bacteriophages. Bacteriophages can potentially be used as an alternative or together with antibiotics, and there is evidence of trade-offs in resistance for some combinations[97,98]. Comparatively few theoretical studies have considered the effect on coexistence of other ecological processes that are thought to be a common feature of microbial systems, such as cross-feeding and direct competition (but see ref. [99]), but it is again straightforward to incorporate these dynamics into classic models of resource competition. For example, using an individual based competition model, one study[100] found that cross-feeding weakened the negative impacts of antibiotics on sensitive strains. Similarly, the theoretical literature on antibiotic resistance should provide a wealth of examples for how other evolutionary and physiological factors (for example, horizontal gene transfer, mutation rates and an active immune system) might be incorporated into the kinds of ecological models we have focused on here.

Conclusions

The motivation behind this Perspective was to present recent concepts from theoretical ecology that could prove useful in understanding the evolution of antibiotic resistance and the coexistence of susceptible and resistant pathogens in microbial communities. It remains to be seen whether these insights can ultimately be of use in developing strategies to manage resistance. As with much theoretical knowledge, the path to generality is littered with simplifying assumptions that still need to be tested and verified empirically. Nevertheless, with increasing awareness that antibiotic-resistant pathogens are embedded within complex species interaction networks, it would be short-sighted not to take advantage of a wealth of valuable theory and models at the frontier of community ecology.

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Author contributions

A.D.L., A.R.H. and J.M.L. conceived the study. A.D.L. performed simulations and analysis. A.D.L., A.R.H. and J.M.L. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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