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SPECIAL ISSUE

THE RISE OF RESISTANCE

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RESISTANCE

By **Caroline Ash**

Almost as soon as antibiotics were discovered to be valuable in medicine, resistance emerged among bacteria. Whenever mutating or recombining organisms are faced with extirpation, those individuals with variations that avert death will survive and reproduce to take over the population. This can happen rapidly among organisms that reproduce fast and outpace our efforts to combat them. Thus, our use of chemical entities to rid ourselves of clinical, domestic, and agricultural pathogens and pests has selected for resistance.

Today, we find ourselves at the nexus of an alarming acceleration of resistance to antibiotics, insecticides, and herbicides. Through chemical misuse, resistance also brings widespread collateral damage to natural, social, and economic systems. Resistance to antifungal agents poses a particular challenge because a limited suite of chemicals is used in both agricultural and clinical settings.

Evolution will always circumvent head-on attack by new biocides, and we may not be able to invent all the new products that we need. We must therefore harness evolutionary approaches to find smarter ways to minimize the erosion of chemical susceptibility. We now have it in our means to integrate a variety of approaches to pest and pathogen management, including rigorous regulation of prescription behavior, consistent use of clinical hygiene measures, physical barriers to crop pests, and alternative cropping regimes. We urgently need to revisit our reliance on chemicals to ensure our future medical and food security.

A farmer sprays pesticides on crops. Our health and food security are threatened by escalating resistance to such biocides.

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REVIEW

Wicked evolution: Can we address the sociobiological dilemma of pesticide resistance?

Fred Gould,[1,2]* Zachary S. Brown,[1,3] Jennifer Kuzma[1,4]

Resistance to insecticides and herbicides has cost billions of U.S. dollars in the agricultural sector and could result in millions of lives lost to insect-vectored diseases. We mostly continue to use pesticides as if resistance is a temporary issue that will be addressed by commercialization of new pesticides with novel modes of action. However, current evidence suggests that insect and weed evolution may outstrip our ability to replace outmoded chemicals and other control mechanisms. To avoid this outcome, we must address the mix of ecological, genetic, economic, and sociopolitical factors that prevent implementation of sustainable pest management practices. We offer an ambitious proposition.

The first documentation of resistance evolving to an insecticide was published in 1914, and the researcher who discovered the problem emphasized that if we did not develop approaches for more judicious use of insecticides, the problem of resistant pests would continue (*1*). Although agriculturalists have developed the field of "resistance management," with more than 3000 publications since 1980 (*2*), we mostly continue to use insecticides and herbicides (hereafter collectively called pesticides) as if resistance is a temporary issue that will be solved by commercialization of new products with novel modes of action (*3*). Evolution of resistance by arthropods and weeds to control measures costs billions of U.S. dollars per year (*4*, *5*) and may lead to loss of millions of lives (*6*). Breakthroughs in chemistry and molecular biology may provide many new pesticides and novel methods for pest control, but there is also a considerable chance that the evolution of pest resistance will outpace human innovation.

Consider the case of malaria, where the use of insecticide-treated bednets (ITNs) and indoor residual sprays (IRS) is estimated to have averted more than 0.5 billion cases of malaria between 2000 and 2015 (*7*). Resistance is evolving to the insecticides used, and there is growing concern over resurgence of the malaria-vector mosquito populations (*6*). Although efforts are being made to develop new insecticides aimed at mosquitoes (*8*), it is not clear that the new compounds will become available soon enough and be as cost-effective as the current ones.

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In 1996, companies commercialized genetically engineered crops that were not harmed by glyphosate, an herbicide that has broad-spectrum toxicity to weed species. The flexibility and profits that these crops brought to farmers resulted in over 90% of U.S. maize (corn), soybean, and cotton hectares planted to herbicide-tolerant varieties by 2014 (*9*). The accompanying widespread use of glyphosate resulted in more than 40 weed species evolving resistance and consequently diminished the utility of the herbicide-tolerant crop varieties (*10*) (Fig. 1, left). To address this problem, companies have reengineered crops to be tolerant of the plant hormone (auxin)–mimicking herbicides 2,4-D and Dicamba. These herbicides were first commercialized in 1945 and 1967, respectively. This reaching back to the past has become necessary because no herbicides with new modes of action have been commercialized in more than 30 years (*11*). Weed species have evolved resistance to every herbicide class in use (Fig. 1, right), and more than 550 arthropod species have resistance to at least one insecticide (Fig. 2). Cases have emerged where no pesticide remains effective. In Australia, weeds in wheat became resistant to all herbicides available and resulted in farmers designing machines to harvest weed seeds for population suppression [e.g., *12*].

If we are to address this recalcitrant issue of pesticide resistance, we must treat it as a "wicked problem," in the sense that there are social, economic, and biological uncertainties and complexities interacting in ways that decrease incentives for actions aimed at mitigation. Here, we summarize the interacting factors and conclude with a call for government support of ambitious landscape-level experiments to assess which pesticide use strategies decrease resistance risks.

Ecology and genetics

Insecticides and herbicides are typically designed to disrupt or mimic a single biologically active protein that is critical to survival of a pest organism. Protein targets in insects are typically involved in function of the nervous system, but some more recently developed insecticides affect growth and development. Herbicides often target enzymes involved in photosynthesis or growth patterns.

Resistance can emerge from a single mutation making a protein less susceptible to action of the pesticide. Alternatively, a single mutation can increase the amount or efficiency of an enzyme that degrades the insecticide or herbicide. These two modes of resistance are common (*13*, *14*), but other forms of resistance have been found that involve gene duplication or multiple genes acting together, each with a small but additive impact on resistance (*15*).

One or two locus population genetic models permit a general understanding of pesticide resistance evolution. More realistic, predictive models require combining population genetics with empirical data on population biology (e.g., life history, mating behavior, and gene flow) of the pest species and the fitness of each genotype in environments with and without the pesticide (i.e., fitness cost). Accurate data on these parameters are difficult to collect and can vary among localities. Most insecticides are sprayed at a specific concentration on a given crop, but over time the insecticide decays, so insects contacting a sprayed plant 1 day versus 10 days after the spraying encounter different doses. The dose on day 1 might kill 90% of insects homozygous for the susceptible allele and only 10% of those homozygous for the resistant allele, while on day 10, only 20% of the susceptible homozygotes would die. If most of the insects were encountering the insecticide-treated plant on day 1, the rate of resistance evolution would be predicted to be faster than if most of the encounters were on day 10. To further complicate matters with insecticides and herbicides, not every sprayed plant or plant leaf receives the same amount of pesticide. In sexually reproducing weeds and insects, the rate of resistance evolution is strongly influenced by the relative fitness (dominant to recessive) of heterozygotes, and this sometimes depends on the dose of pesticide encountered in the field (Fig. 3). Thus, it is difficult (and controversial) to determine whether resistance is expected to evolve more rapidly to higher or lower application concentrations of a pesticide [e.g., *16, 17*].

Even more complexity arises in attempts to predict resistance evolution when combinations of pesticides are applied (*18*, *19*). Although the idea that such combinations will slow resistance evolution is theoretically controversial and lacks empirical support, mixtures are often recommended at the field level (*15*).

Although there is high uncertainty regarding many resistance management choices, under almost all circumstances entomologists agree that using an integrated pest management (IPM) approach that results in fewer insecticide applications

should decrease the rate of resistance evolution (*18*).

Toxins derived from the bacterium *Bacillus thuringiensis* (Bt) have been widely used in engineered insecticidal crops. Here, variation in the dose of toxin received by insects is less of a problem (*20*). Engineered plants can produce season-long Bt-toxin concentrations that, for some insect pests, kill all susceptible individuals and almost all heterozygotes (*21*). Season-long consistently high toxin doses, when coupled with a percentage of the crop planted to a nontoxic variety (i.e., that act as refuges for susceptibility) is predicted to slow resistance evolution by a factor of 10 to 100. This strategy is known as the high-dose/refuge approach (*17*) and has been used for more than 20 years with some target pests. Tabashnik and Carrière (*22*) have examined 30 cases of long-term planting of Bt-toxin–producing crops: In nine cases where a high dose of Bt was achieved, neither economically important target pest resistance nor early warnings of resistance were found, but in 17 of 21 cases in which high doses were not achieved, resistance had evolved or showed evidence of emergence. Some of the cases of resistance occurred in low- or middle-income nations where refuges were not planted or where the crop varieties were not engineered for the relative susceptibility of the local pests and therefore did not maintain a high enough toxin dose.

The focus in the resistance management literature is on resistance to chemical control, but widespread use of other control tactics—including biological control, crop rotation, and hand weeding—also faces the challenges of resistance evolution (*23*). For example, the northern and western corn rootworms, which are mostly restricted to feeding on maize (corn) roots as larvae, have evolved resistance to the rotation of maize and soybean. One species has evolved to mostly overwinter as an egg for 2 years instead of 1, so when there is a typical 2-year rotation of maize and soybean, the larvae emerge from the hatching eggs in time for the next maize planting. The other species evolved to lay some of its eggs in the soil beneath soybean plants, "anticipating" maize in the next season. Most amazingly, some weeds have evolved to look like rice plants and thus avoid hand hoeing, and others have evolved seeds that mimic those of the crop they infest and are replanted along with crop seeds (*23*).

"...we must treat it [resistance] as a 'wicked problem,' in the sense that there are social, economic, and biological uncertainties and complexities interacting in ways that decrease incentives for actions aimed at mitigation."

Whenever humans act in any way to decrease the fitness of an insect or weed, natural selection is likely to result in a response. Insect growth regulators that mimic hormones were at one time considered resistance-proof insecticides, but in the end this tactic did not deter evolution of resistance (*23*). Ultimately, even with all of the biological uncertainties involved in resistance management, it remains the only current option for limiting the economic and social impact of pest evolution.

Economic perspectives

Pesticide resistance has both economic causes and economic consequences. Agricultural benefits lost from resistance in the United States have been estimated at about US$10 billion per year (*5*). Globally, reliance on pesticides has been increasing (*24*), exacerbating the impact of resistance. Pesticides also bear costs for the environment and public health (*24*). Some pesticides, such as Bt toxins (used either in engineered crops or in organic agriculture), have replaced broader-spectrum pesticides that were more toxic to nontarget organisms (*24*). Hence, a loss in the effectiveness of Bt toxins owing to resistance has environmental consequences if we revert to a less target-specific replacement. This rationale has been used in the formulation of government regulations for managing resistance to Bt crops (*17*).

Insecticide resistance in public health is also imposing substantial damages, although fewer studies are available that quantify the economic costs. Model-based analysis has shown that if disease vector resistance to pyrethroids becomes widespread, cases of malaria averted with ITNs could decline by 40% (*25*). Coupled with the estimate that bednets averted more than 65 million clinical malaria cases in sub-Saharan Africa in 2015 (*7*), and assuming that this figure provides a lower bound for potential cases averted in subsequent years, this would imply around 26 million additional clinical cases of malaria per year as a result of widespread vector resistance. Assuming an approximate lower bound cost of illness of at least $10 per malaria episode (*26*), insecticide resistance could

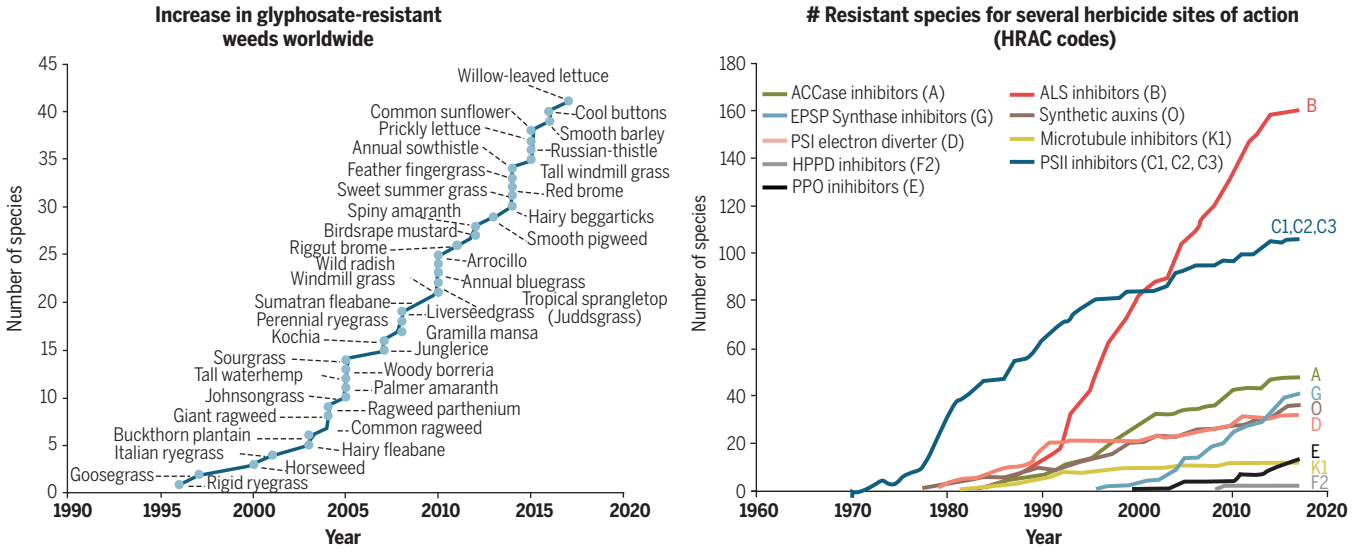

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Fig. 1. Weed species with resistance to herbicides. (**Left**) Cumulative number of weed species with resistance to glyphosate. (**Right**) Cumulative number of weed species with resistance to herbicides in the major mechanism of action groupings.

conservatively cost sub-Saharan Africa at least $260 million per year.

Although these numbers make clear that the potential costs are large enough to warrant stronger policies for managing pesticide resistance, they do not tell us exactly what return society might expect from different investments in resistance management. The most basic insight from economics is that efficient pesticide use should weigh current net benefits of use against the costs of lost future effectiveness (*27*). To assess these future costs, economic discounting and the uncertainty of developing replacement pest control technologies must be factored in. As yet, the user costs of resistance are not computed in any systematic way, although recent methods for computing prices for natural capital and ecosystem services could be applied (*28*).

Laxminarayan and Simpson (*29*) have analyzed the optimal refuge sizes for managing pest resistance to Bt crops. They found that fitness costs of resistance are critical for determining whether refuges are economically efficient in the long run. Fitness costs determine whether susceptibility can be renewed after accumulating high levels of resistance in the pest population. If this renewal rate is less than an expected rate of return on financial assets, then it is optimal in the long run to deplete pesticide susceptibility. Likewise, the importance of fitness costs has been shown for economic management of resistance to pyrethroid insecticides in malaria control (*30*) and agriculture (*31*).

Fitness costs, dominance, and initial frequencies of resistance genes remain highly uncertain in field settings for many pesticides. However, reducing uncertainty is costly, and better information may be more actionable for some of these factors than others, as has been shown for malaria vectors (*32*). For example, more certainty about the efficacy of noninsecticidal alternatives may be more valuable than better information about the fitness costs of resistance.

Ultimately, the costs of pesticide resistance to users depend on available control alternatives. However, no herbicides with new modes of action have been commercialized in more than 30 years, and the estimated cost of discovery of new insecticides has increased by a factor of eight in the past 50 years (*33*). Other tools with demonstrated effectiveness at managing resistance within an IPM framework range from biocontrol (*34*) to the sterile insect technique (*35*), but the implementation of these approaches is costly and complicated.

Pesticide susceptibility shares properties of a common pool resource (*36*). One party's use of a pesticide draws down the stock of susceptibility to that pesticide available not only to that party but also to other users. Furthermore, one user cannot limit use of the stock by others. The result is that users overexploit the resource relative to what would be economically efficient. One solution is to tax pesticide use to reflect the marginal user costs of resistance and the negative environmental impacts of pesticides. Four European countries have implemented pesticide taxes based on these motivations, although practical challenges impede their broader adoption (*37*).

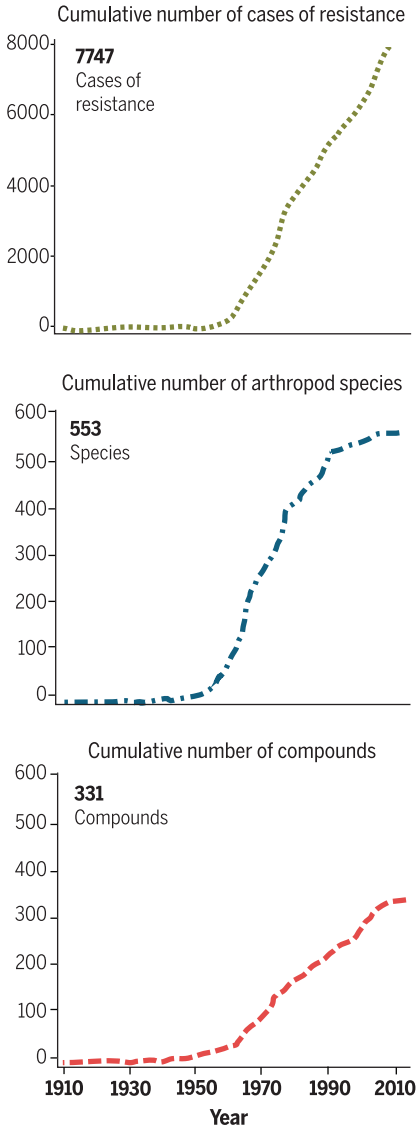

Fig. 2. Arthropods with resistance to insecticides. Data from 1910 to 2010 showing total number of species (dark blue dotted and dashed line), total number of cases of resistance to any insecticidal compound reported from a new location (green dashed line), and total number of compounds with resistance found in at least one arthropod species (light blue dashed line) (*56*).

One rationale supporting the laissez-faire management of weed resistance to glyphosate was the erroneous assumption that weeds were relatively immobile (*3*). This contrasts with extensive regulation of Bt crops to manage insect resistance, where the mobility of target pests of Bt crops was explicitly used as one rationale in refuge policies (*17*).

Because the use of Bt crops and other control tactics can result in suppression of the target pest over wide areas, incentives for overexploitation of susceptibility can be counterbalanced by the public good of areawide pest suppression. For example, areawide suppression of the European corn borer in the U.S. Midwest from use of Bt maize reduced pest damages by $2.4 billion among growers of non-Bt maize (*38*). Subsequent modeling shows that this areawide protection incentivizes planting of non-Bt varieties (*39*), which is predicted to slow resistance evolution further.

Sociopolitical perspectives

Efforts to decrease the uncertainties of pest resistance are critical to effective management, but an understanding of how these aspects intersect with social and political factors is also needed. Currently, the emphasis is on educational and incentive programs. However, these have not substantially improved resistance management and, as Ervin and Jussaume explain, "often fail to take into account the fact that farm-level decision-making takes place within complex social-cultural settings" (*40*). Sociopolitical research in this area applies at the level of the individual (micro level), the community (meso level), and the federal government or nation-state (macro level). Sociopolitical approaches have rarely been applied to resistance management, so concepts and examples must be drawn from other settings.

Individual level

The individual level of decision-making about pesticide use and resistance management mostly resides with farmers. In public health, households are often the key micro-level decision-makers, as in the case of whether or how to use a bednet. Most research on individuals' perceptions and decisions about pesticide use is framed around economic models of demand for pest control and risk reduction (*41*, *42*) and does not specifically address resistance. Resistance management could benefit from risk perception studies that have been used to analyze other technologies. Such studies would shed light on how factors associated with (i) technological options (e.g., controllability and familiarity), (ii) individuals themselves (e.g., culture, demographics, and worldviews), or (iii) risk managers and communicators (e.g., level of trust and perceived fairness) influence people's perception of risk and motivate them to take action for reducing resistance.

Community level

At the community level, social systems can support or interfere with resistance management programs and compliance. Social capital has been correlated with positive effects on IPM and sustainability, especially in developing nations (*43*). Research on network ties and social capital among U.S. farmers, and their relationship

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to the successful implementation of resistance management programs, could shed light on how to enhance collective action.

Because pest susceptibility can often be considered a common pool resource, Ostrom's work on the governance of such resources suggests that resistance may sometimes be better managed by on-the-ground, networked communities generating their own rules and norms for pesticide use (*44*) than by more formal, top-down governance. Regional programs, such as weed management areas, in which local farmers vote to implement different resistance management strategies (*40*), fit this model. In another example, pink bollworm resistance to Bt cotton in the southwest United States has been effectively delayed through voluntary cooperative initiatives and cost-sharing between regional grower associations and the U.S. Department of Agriculture (*35*). In terms of management tools, policy process frameworks, such as institutional analysis and development, can inform the design, implementation, and evaluation of common pool resource governance systems (*44*, *45*). Behavioral tools, such as social marketing, to engender norms for resistance management have also shown recent promise (*46*), but further research is needed.

Macro level

Systems theory and thinking at the macro level can help to uncover the underlying factors contributing to policy problems, such as resistance management, by taking complexity and multiple types of competing and intersecting forces into account (*47*). In complex situations, quite often the most intuitive policies have immediate benefits but over time exhibit counterintuitive behavior (i.e., policy resistance) and fail owing to unanticipated feedback (*48*). For example, the price of maize rose in the first decade of the 21st century in large part due to ethanol mandates in midwestern states, as well as subsidies and higher oil prices. This led to a near-term economic advantage for farmers who stopped rotating maize with soybeans and instead planted maize continuously (*49*). The continuous planting of Bt maize could have led to higher pest resistance to Bt in those areas, an issue that requires further investigation.

Political economy studies at the macro level can also uncover underlying tensions and barriers to effective solutions. For example, chemical companies will desire to sell more pesticides and increase short-term company profits. Sales tactics will compete with government regulators' desires to contain pesticide use to mitigate health and environmental risk. However, recognizing the need to protect the efficacy of their products over the long term, some biotechnology companies selling Bt crop seed have partnered with federal agencies and farmers to implement resistance management programs. For instance, the selling of seed bags with a mixture of Bt and non-Bt seeds allows companies to maintain their level of product sales while complying with regulatory guidelines. It also improves compliance by farmers, although it decreases a farmer's ability to control the situation and might therefore increase their perception of risk and decrease trust at the micro level.

"Lacking data from bold experiments, we will likely just learn that heavy use of 2,4-D and Dicamba results in weed resistance and that we have an even more critical need for herbicides with new modes of action."

National research policy affects how much knowledge and data we have on all of the factors relating to pest resistance and management. Gaps in biological and economic research are affected by the national priorities of each political administration but have traditionally been underresourced, despite their importance to the growing challenge of resistance management (*50*).

A way forward?

We have seen how pesticide resistance is a "wicked problem" arising from interacting uncertainties and competing interests that decrease incentives for action. A pessimistic conclusion would be that the status quo of little action will hold until a major crisis arises. A more proactive stance is challenging but likely to be less costly in the long run, so we conclude by suggesting two optimistic ways forward.

First, in the case of engineered insecticidal crops, a natural experiment has already been performed, and we know with some certainty what action needs to be taken to develop high-dose/refuge approaches that when tailored to specific systems will slow resistance evolution. Still, we must overcome competing interests that hinder our ability to build the political will on the part of governments to work with companies and farmers to ensure appropriate development and use. As observed by Foley (*51*), "GMOs [genetically modified organisms] have

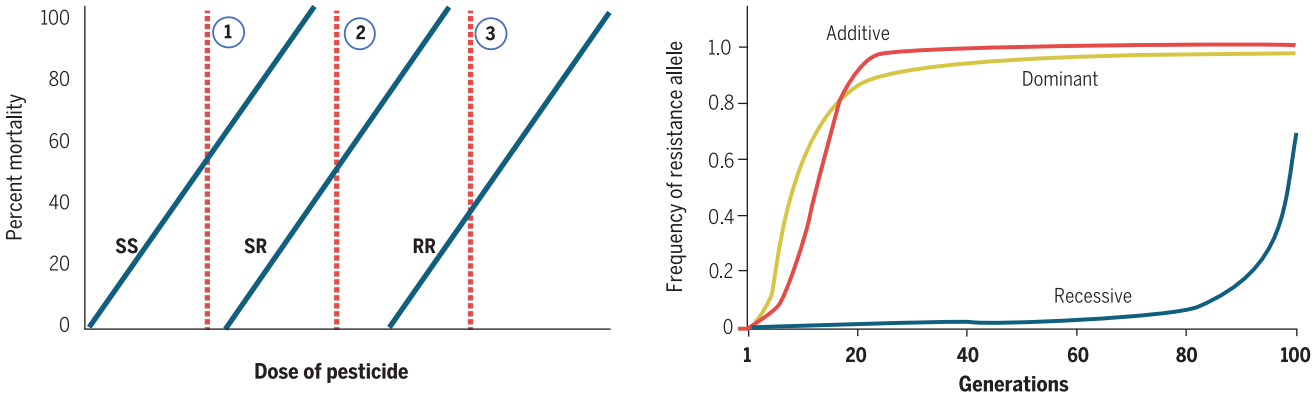

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Fig. 3. Response to selection for resistance to toxins. (**Left**) The solid dark blue lines show the generally expected relationship between the dose of toxin and the mortality of pests that are homozygous for susceptibility alleles (SS), heterozygous (RS), and homozygous for resistance (RR). The vertical, dashed red lines (numbered 1, 2, and 3) show the expected mortality of the three genotypes at different toxin doses. At dose 1, the RS and RR individuals similarly have no mortality, whereas the SS individuals have 50% mortality, so the resistance trait is dominant. At dose 2, the RS mortality is intermediate between SS and RR, so resistance is additive. At dose 3, there is 100% mortality of SS and RS and only 30% mortality of RR, so resistance is recessive. (**Right**) Trajectories of increase over time in resistance allele frequency when resistance is dominant, additive, and recessive.

frequently failed to live up to their potential, not because they are inherently flawed, but because they have been deployed poorly into the complex social and environmental contexts of the real world." Governments should insist on feasible plans for strict enforcement of appropriate use as a condition for commercialization. Knowledge from the social and natural sciences will be needed to guide such governance.

The second and more complex challenge to tackle is for conventional pesticides where there is still a high degree of uncertainty about what the best approaches are to stymie resistance. Although we have data from small-scale experiments, these are not sufficient for understanding resistance dynamics at a landscape level. For crop insects and weeds, large-scale, experimental agriculture, coupled with technical innovation, must go hand in hand. New breakthroughs in genomics and bioinformatics are providing tools that enable detection of genomic responses of insects and weeds to selection with pesticides [e.g., (*52*)]. These tools will put us in a good position to conduct landscape-level experiments on the order of thousands of hectares to decrease uncertainty about the effectiveness of various resistance management practices. It should be possible to detect early genomic and biological signs of resistance and to change management practices before resistance becomes an economic problem. Although these measures will be expensive, complex experiments even with the most localized pests, similar, large-scale endeavors have been tried for eradication of specific insects and weeds, so some of the groundwork has been laid. In addition, such studies will require input from the social sciences to gain appropriate community involvement. Although large-scale experimentation is a substantial investment, in the United States the cost to the federal government (i.e., to taxpayers) for crop insurance to cover crop failures in 2011 was estimated at more than $11 billion, with 265 million acres enrolled (*53*). Policies are being pursued to encourage other agricultural practices, such as cover crops for soil conservation, by tying cover-crop planting to discounts on crop insurance premiums (*54*). Similar approaches could be used for pesticide resistance management. The United States is not the only country with crop subsidies. Certainly, there is a way to use these public investments for the public good of avoiding the long-term costs of resistance.

The United States is about to begin a huge experiment with the commercialization of engineered crops resistant to the action of 2,4-D and Dicamba. These two herbicides will likely be used alone and in combination with glyphosate, despite a lack of knowledge about what usage pattern would be best for decreasing the emergence of resistance in weed populations while maintaining economic viability. This ignorance is reflected in the literature from the EPA and companies that simply tells farmers that diversified approaches to weed management are best for delaying resistance, but with no supporting evidence or incentives (*55*).

Governments and universities could adopt incentive systems to create landscape-level experiments to test different spray combinations, rotations, or combined cultural and chemical controls on large acreages. Genomic responses of weeds would be monitored carefully enough to eliminate any failed strategy before troublesome resistance evolved. Setting up such experiments would require large investments and highly skilled management of people and technologies. This may seem radical, but governments do make similar investments to decrease erosion, maintain conservation reserve programs, and subsidize crop-loss insurance. Lacking data from bold experiments, we will likely just learn that heavy use of 2,4-D and Dicamba results in weed resistance and that we have an even more critical need for herbicides with new modes of action.

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REVIEW

Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens

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Whole-genome sequencing (WGS) has been vital for revealing the rapid temporal and spatial evolution of antimicrobial resistance (AMR) in bacterial pathogens. Some antimicrobial-resistant pathogens have outpaced us, with untreatable infections appearing in hospitals and the community. However, WGS has additionally provided us with enough knowledge to initiate countermeasures. Although we cannot stop bacterial adaptation, the predictability of many evolutionary processes in AMR bacteria offers us an opportunity to channel them using new control strategies. Furthermore, by using WGS for coordinating surveillance and to create a more fundamental understanding of the outcome of antimicrobial treatment and AMR mechanisms, we can use current and future antimicrobials more effectively and aim to extend their longevity.

When antimicrobial drugs were introduced into clinical usage in the mid-20th century, they had an astonishing impact on human health. Infectious bacteria that had threatened our survival were now at the mercy of a chemical arsenal. Previously fatal infections, from whooping cough and scarlet fever to tuberculosis and syphilis, were no longer considered a threat. Antimicrobials substantially reduced the risks associated with child birth, injuries, and invasive medical procedures. What has followed in the subsequent 70 years or so has been an uncontrolled microbiological experiment conducted on an unprecedented scale. Initially we identified a plethora of new antimicrobial classes targeting different essential bacterial functions, but we deployed them haphazardly in ever-increasing quantities. Now antimicrobial resistance (AMR) poses a genuine threat to human health, with the potential to return us to a situation where common infections are as untreatable as they were in the pre-antimicrobial era (*1*).

Humans did not create AMR; we simply promoted it by applying evolutionary pressure. Almost all antimicrobials have chemical similarities with compounds that can be found naturally; AMR genes have been found deep in the permafrost (*2*) and arose long before humankind's ability to synthesize antibacterial chemicals and use them en masse. Therefore, AMR in bacterial populations is a largely predictable phenomenon; the more commonly a specific antimicrobial compound is used, the more likely it is that resistance will emerge and be maintained in an exposed microbial population. The specific dynamics of the processes associated with AMR are, however, less predictable. The rapidity with which diverse AMR phenotypes have emerged and become established within human, animal, and wider environmental populations of microbes has been astonishing and most likely accelerated by concurrent advances in human development, mobilization, and population growth.

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"The first reports of penicillin-resistant infections occurred early in the 1940s, but a penicillinase was described even before the continued clinical usage of the prototype antibiotic."

The evolutionary dynamics of antimicrobial resistance

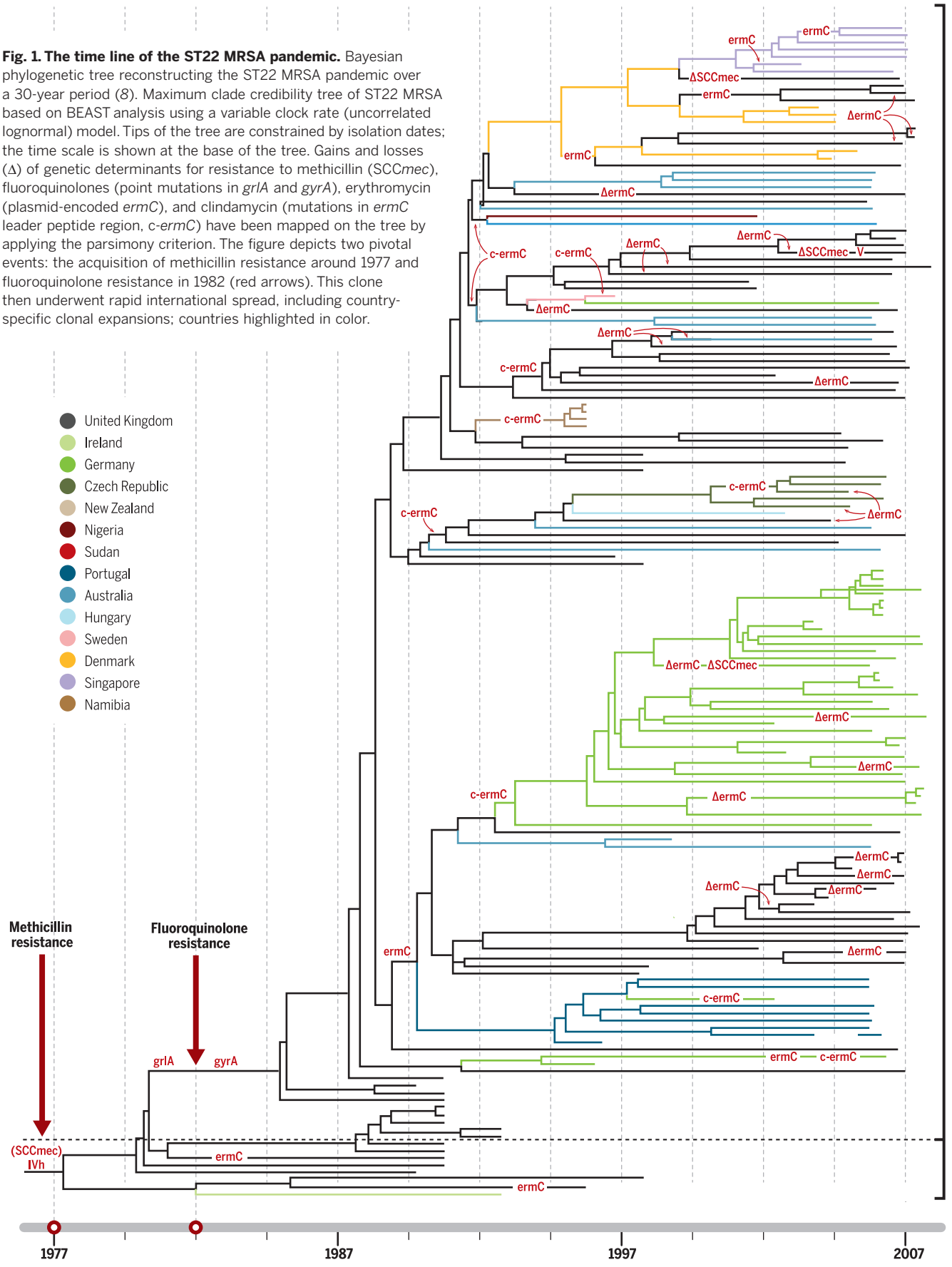
How resistance is maintained and distributed within bacterial populations is a function of the organism's lifestyle (i.e., transmission mode, colonization, and pathogenicity) and the genetic basis for resistance, which can be either intrinsic (i.e., the organism naturally lacks the specific pathway targeted by the drug), mutation associated (i.e., induced changes are passed vertically to descendants), or acquired via horizontal gene transfer (HGT) between organisms (with acquired genes then being passed vertically to progeny). The first reports of penicillin-resistant infections occurred early in the 1940s, but a penicillinase was described even before the continued clinical usage of the prototype antibiotic (*3*). Since then, there have been numerous examples of the rapid emergence of bacteria exhibiting resistance to a specific antimicrobial class soon after its introduction (*4*). However, in the past decade, through the advent of high-throughput whole-genome sequencing (WGS), we have been able to make substantive advances in understanding the dynamics of AMR evolution and spread in bacterial populations.

WGS has become the key technology for understanding pathogen evolution, population dynamics, and genomic epidemiology, as it provides a far greater degree of reproducibility, standardization, and resolution than previous genotyping methods (*5*). By capturing both the neutral evolution of the population—for tracking transmission and diversification of the organism—and the genetic determinants of AMR, WGS can reveal detailed temporal and spatial dynamics of AMR evolution and simultaneously infer the impact of AMR selection on pathogen populations. Much of the pioneering WGS-based AMR work was focused on the opportunistic Gram-positive human pathogen *Staphylococcus aureus*, particularly with respect to the emergence of methicillin resistance (MRSA) in health care facilities in Europe (*6*). MRSA is still among the best examples of how AMR variants can rapidly emerge, be efficiently maintained, and spread at different spatiotemporal scales, ranging from individual hospital wards to health care networks, and internationally within human populations (Fig. 1). MRSA was first observed in 1960, within a year of the introduction of second-generation β-lactams, such as methicillin, into clinical practice. However, phylogenetic reconstruction showed that MRSA actually emerged in the 1940s via HGT of the staphylococcal cassette chromosome *mec* (SCC*mec*) element, as a consequence of the initial mass usage of penicillin (*7*). WGS data shows that MRSA has arisen on numerous occasions independently in different subpopulations on different continents (e.g., USA300, ST22 in Europe, and ST93 in Australia) through parallel HGT events and spread throughout health care systems (*6*). The history of health care–associated MRSA in the later part of the 20th century was punctuated by frequent epidemics associated with highly successful clones, such as EMRSA-15 (ST22), which was first described in the United Kingdom in the 1990s and then spread throughout Europe, and then intercontinentally (Fig. 1) (*8*). Notably, a fluoroquinolone-resistant EMRSA-15 variant arose in the United Kingdom soon after clinical trials with ciprofloxacin in the 1980s, with point mutations in the DNA gyrase and topoisomerase IV genes. This critical event was the apparent trigger for the subsequent pandemic spread of a fluoroquinolone-resistant MRSA variant (Fig. 1) (*8*).

The global dissemination of antimicrobial-resistant clones

MRSA epitomizes a now all-too-familiar evolutionary route by which successful AMR clones

Fig. 1. The time line of the ST22 MRSA pandemic. Bayesian phylogenetic tree reconstructing the ST22 MRSA pandemic over a 30-year period (*8*). Maximum clade credibility tree of ST22 MRSA based on BEAST analysis using a variable clock rate (uncorrelated lognormal) model. Tips of the tree are constrained by isolation dates; the time scale is shown at the base of the tree. Gains and losses (Δ) of genetic determinants for resistance to methicillin (SCC*mec*), fluoroquinolones (point mutations in *grlA* and *gyrA*), erythromycin (plasmid-encoded *ermC*), and clindamycin (mutations in *ermC* leader peptide region, c-*ermC*) have been mapped on the tree by applying the parsimony criterion. The figure depicts two pivotal events: the acquisition of methicillin resistance around 1977 and fluoroquinolone resistance in 1982 (red arrows). This clone then underwent rapid international spread, including country-specific clonal expansions; countries highlighted in color.



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emerge in response to local antimicrobial usage, undergo population expansion under selection from sustained antimicrobial exposure, and then explode into pandemic spread. The finer details are organism specific and dependent on their particular evolutionary landscape (e.g., mechanisms of resistance, fitness costs, modes of transmission, and host range), but all follow a similar basic trajectory, mirroring that observed in the recent MEGA-plate experiment (*9*). Briefly, exposure of susceptible bacteria to antimicrobial drugs will result in the local emergence of resistant mutants. This happens continuously, as a genetically diverse pool of pathogens are exposed to a range of different compounds at different concentrations. Most resistant mutants will be purged quickly from the population, either through genetic drift or because they are less fit for onward transmission. For example, WGS data have shown that that a few common resistance mutations emerge repeatedly in *Mycobacterium tuberculosis* during the treatment of individuals but that these are rarely transmitted (*10*). However, occasionally a resistant mutant will have a sufficient fitness advantage to undergo local clonal expansion in a subset of infections. This occurs through a combination of ongoing antimicrobial exposures and/or a genetic background that moderates the fitness cost, e.g., the compensatory mutations in rifampicin-resistant *M. tuberculosis* (*11*); the increased replication rate of *Salmonella* Typhi with fluoroquinolone resistance–associated DNA gyrase mutations (*12*); or chromosomal variants that ameliorate the cost of AMR plasmid carriage (*13*). Once established, the locally successful AMR clone may face opportunities for further expansion, including potentially broader geographical dissemination and/or spillover into other host populations, depending on the mode of transmission and the extent of antimicrobial selection it encounters.

WGS investigations show that clonal expansion and ensuing geographical dissemination of pathogens can mostly be traced to the acquisition of a specific AMR determinant(s) like SCC*mec* in MRSA. This suggests the AMR element(s) function as the "king maker" within the various pathogen populations, determining which clones dominate locally, regionally, and globally. Some mobile AMR genes have played this role in multiple organisms and clones; e.g., CTX-M-15 has driven the success of *Escherichia coli* ST131 and several *Klebsiella pneumoniae* clones (CG14/15, ST101) (*14*, *15*). Equally, AMR genes also benefit by association with certain plasmid vectors or host bacterial clones, which act as vehicles for dissemination. *K. pneumoniae* is host to several key mobile AMR genes and has played a pivotal role in the global dissemination of various extended spectrum β-lactamases (ESBLs) and the carbapenemases KPC and NDM-1 (*15*). This association may be linked to *K. pneumoniae*'s broad ecological range and propensity for HGT, which provide a conduit for AMR gene trafficking from a very large gene pool into the smaller subpopulations of human-associated bacteria.

Another common reoccurring observation is the accumulation of additional resistance mechanisms in an already established AMR clone, such as fluoroquinolone resistance in EMRSA-15 (*8*). This phenomenon is likely driven by escalating antimicrobial use to tackle AMR infections, accompanied by a relaxation of selective constraints and an increased effective population size of the successful clone. It is particularly common in organisms that can accumulate multiple AMR genes through HGT, particularly within the Enterobacteriaceae (*14*, *15*), but is also evident in the highly clonal and evolutionarily constrained *M. tuberculosis*, in which resistance to isoniazid via a mutation in *katG* commonly precedes further AMR mutations (*10*).

Health care–associated "superbugs"

AMR organisms are highly destructive in hospitals. Modern medicine relies on antimicrobial therapy and prophylaxis to protect against opportunistic infections, which affect approximately 1 in 10 hospitalized patients globally. In industrialized countries, health care–associated infections account for the vast majority of the communicable disease burden (*16*), but hospitals on all continents are now plagued by AMR infections. The combination of intensive antimicrobial exposure in hosts whose immune systems are struggling to defend against infecting bacteria can rapidly select for resistance. Several WGS studies have documented local emergence of resistance in hospitalized patients in response to specific antimicrobial exposures, which have been studied in individual infections, treatment episodes (*17*), and at the ward level (*18*). These studies show that many of the same mutational events arise repeatedly in different patients and in different host backgrounds, demonstrating that the emergence of AMR in many organisms within health care facilities is often predictable. Examples include the repeated acquisition of SCC*mec* (methicillin resistance), *walKR* mutations (vancomycin resistance) in *S. aureus* (*18*), and *lpx* disruptions (colistin resistance) in *Acinetobacter baumannii* (*17*).

Although AMR organisms arise continuously, national- and international-level WGS snapshots show that most AMR infections are attributable to a few clones within the broad population of the specific pathogen. Thus, only a small fraction of emergent AMR variants is sufficiently fit for broader dissemination. WGS investigations of Gram-negative opportunistic pathogens mimic the pattern of MRSA, with clonal spread that begins as localized expansions, rapidly progressing to intercontinental spread (within years) and even global dissemination (within decades). Particularly concerning is *K. pneumoniae* clone ST258, which carries the plasmid-borne *K. pneumoniae* carbapenemase gene *KPC* that confers resistance to all β-lactams, including carbapenems and cephalosporins (*15*). KPC ST258 arose in the United States, where it began causing hospital outbreaks around 2005. After first spreading to Israel, by 2009, KPC ST258 was endemic in Greece and Italy and has since spread across Europe and South America and into Asia and Australia (Fig. 2). The arrival of the clone in new locations is linked to patients with a history of recent international travel to KPC ST258–endemic areas. Other carbapenemase-producing *K. pneumoniae* clones have also emerged (e.g., OXA-48 ST405 in Spain and KPC ST11 in China), but these have remained relatively localized. Why a combination of the *KPC* gene in the ST258 *K. pneumoniae* host background has been so successful remains an important unanswered question.

Other relevant Gram-negative health care–associated AMR clones include the ESBL-producing *E. coli* ST131, whose global dissemination has been so rapid that its initial geographical origins were obscured (*14*). *A. baumannii* Global Clone 1 (GC1) is probably the oldest multidrug-resistant (MDR) hospital clone of *A. baumannii* and emerged in the 1980s after acquisition of a genomic island conferring resistance to all first-line antimicrobials. GC1 latterly accumulated resistance against fluoroquinolones and carbapenems (*19*). The prevention and management of infections with these highly resistant clones is a major health care challenge, and alternative strategies, such as vaccines and targeted immunotherapies, are urgently needed. However, *K. pneumoniae* KPC ST258, *E. coli* ST131, and *A. baumannii* GC1 all display extensive surface antigen diversification, complicating such approaches (*15*, *19*).

Antimicrobial resistance in community-acquired infections

AMR is not only a substantial problem in health care systems but is also prevalent among a wide range of pathogens associated with community-acquired infections. WGS studies show that AMR in the community setting, as in hospitals, is similarly dominated by a small number of globally disseminated clones that have accumulated AMR determinants over time. The waterborne enteric diseases typhoid fever and dysentery provide two salient examples. The vast majority of MDR typhoid fever cases globally are caused by the *Salmonella* Typhi H58 clone, which emerged in South Asia in the early 1990s in association with an MDR plasmid and has since spread throughout Asia and into East Africa, accumulating fluoroquinolone resistance mutations in the genes encoding DNA gyrase and topoisomerase IV (Fig. 2) (*20*). Most pediatric cases of MDR shigellosis are caused by a *Shigella sonnei* clone that carries a mobile genetic element conferring resistance to almost all first-line drugs on its chromosome. The clone emerged in the 1970s and is now globally disseminated (Fig. 2) (*21*), with the same fluoroquinolone resistance mutations as in *S.* Typhi arising subsequently and spreading out from South Asia (*22*).

Sexually transmitted infections (STIs) present particular complications for understanding AMR in community-acquired disease, as their transmission is driven by complex human behavior. AMR in STIs share the same general evolutionary characteristics as AMR in health care–acquired infections, but have distinct transmission, diagnosis, and treatment parameters that result in distinct spatiotemporal dynamics. AMR in STIs are a major concern; data from locations with good STI surveillance systems suggest a general upward trend in bacterial STI incidence disproportionately associated with specific communities (*23*). In 2014, men-who-have-sex-with-men (MSM) represented <2% of the London adult population; however, 28% of all new STIs were diagnosed in this community. More specifically, 69% of all new cases of gonorrhea diagnosed in London were in MSM, and the emergence of some AMR variants of *Neisseria gonorrhoeae* have been specifically linked to MSM communities (*23*). AMR in *N. gonorrhoeae* is such a potential problem that it has been acknowledged by the World Health Organization (WHO) as being a major threat to human health (*24*). MDR variants leave increasingly limited treatment options, and there is a very real prospect of widespread resistance to ceftriaxone, the last remaining option for empirical monotherapy. Indeed, there have already been isolated reports of *N. gonorrhoeae* that exhibit resistance to all current treatments (*24*). One of the first epidemiological studies exploiting WGS for *N. gonorrhoeae* aimed to understand how particular AMR phenotypes had emerged. This study showed that reduced susceptibility against third-generation cephalosporins in the United States between 2000 and 2014 was the consequence of the expansion of two particular clones arising within the MSM community that possessed the mosaic *penA* resistance allele (*25*).

For other STIs, the situation is less clear. Despite reports of mutations in *Chlamydia trachomatis* conferring in vitro resistance against macrolides (the first-line treatment for chlamydia), there is no evidence for the stable maintenance of these mutations during human infection (*26*). Similarly, intramuscular injection with benzathine penicillin appears to remain generally effective for treating syphilis (*Treponema pallidum*). However, we are missing key epidemiological information on many STIs. In well-resourced clinical settings, there is a move away from microbiological culture as the "gold standard" for the diagnosis of bacterial STIs and increasing reliance on molecular testing (*24*). Although molecular tests have the advantage of being rapid and sensitive, they have the disadvantage of being destructive and do not screen for potential AMR phenotypes (*24*). This situation is exacerbated in resource-limited settings where any form of diagnostic testing is rare, which results in a substantial underreporting of STIs and almost no AMR or pathogen prevalence data (*24*).

"Sexually transmitted infections present particular complications for understanding AMR in community-acquired disease, as their transmission is driven by complex human behavior."

Another issue complicating AMR detection in STIs is the challenge of individual case management. A lack of diagnostic testing imposes a reliance on empirical syndromic therapy, which can have undesired consequences for driving the emergence of new AMR-STIs because of undirected antibiotic treatment. *Shigella* spp. are fecal-oral pathogens with a notoriously low infectious dose and are adept at acquiring new functions via HGT. *Shigella* has emerged as an enteric STI with a capacity for global dissemination of AMR genotypes. *Shigella* outbreaks in MSM communities have been sporadically observed since the 1970s (*27*). An increase in MSM-associated dysentery has been reported recently in the United Kingdom with a *Shigella flexneri* resistant to azithromycin in individuals with no history of travel to countries with highly endemic *Shigella* (*28*). Azithromycin is not routinely used to treat dysentery in the United Kingdom, but is the front-line treatment for gonococcal urethritis, syphilis, and chlamydia. The emergence of this *S. flexneri* variant was linked to the acquisition of a conjugative plasmid carrying various macrolide resistance genes, which was likely driven by azithromycin treatment for other STIs. Transmission of organisms via oro-anal sex, coupled with HIV-associated immunodeficiency, multiple sexual partners, and greater

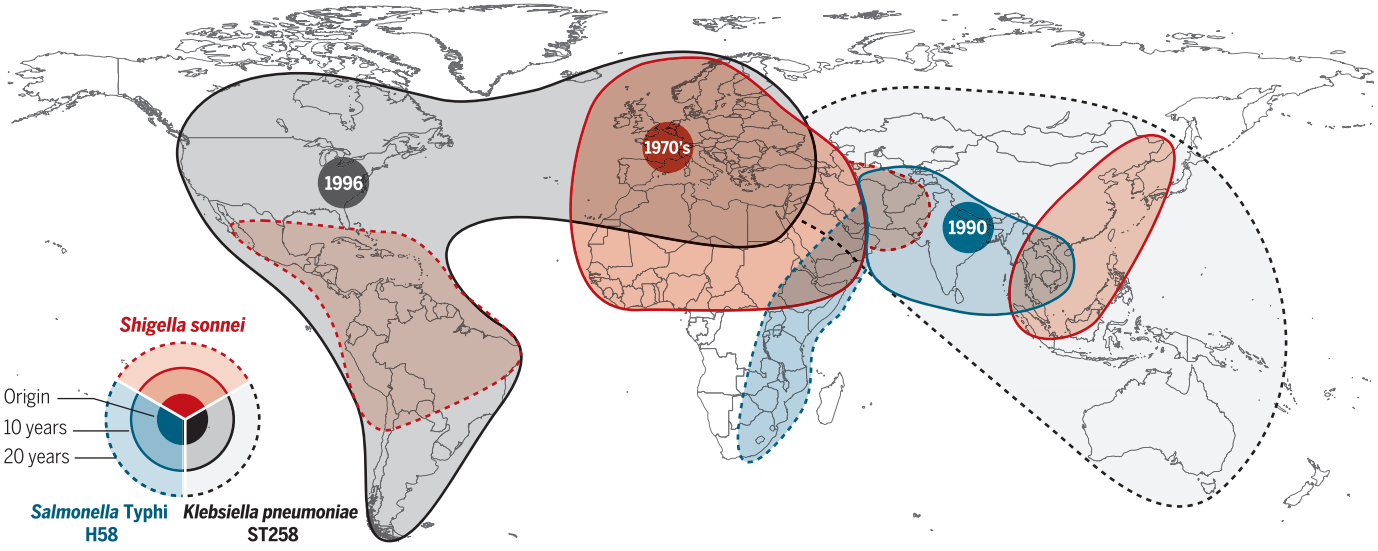

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Fig. 2. Origin and blast radius for the clonal expansion for three multidrug-resistant Gram-negative bacteria clones. The map summarizes data for the global dissemination of: dysentery causing *Shigella sonnei* clone lineage III-global, with a chromosomal insertion of a mobile genetic element encoding resistance to streptomycin, trimethoprim-sulfamethoxazole, and tetracycline (red); the typhoid fever pathogen *Salmonella* Typhi, clone H58, with a plasmid encoding resistance to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, streptomycin, and tetracycline (blue); health care–associated *Klebsiella pneumoniae* clone ST258, carrying the KPC carbapenemase encoding resistance to all β-lactam antimicrobials, including carbapenems and third-generation cephalosporins (gray).

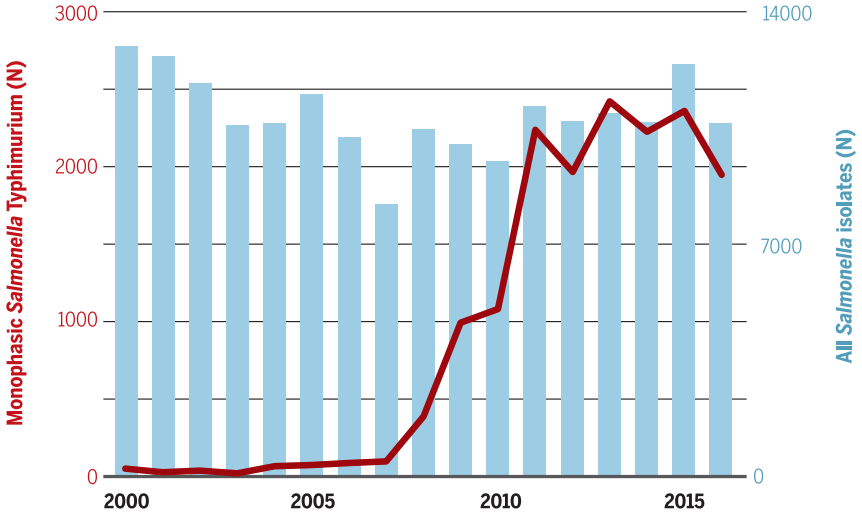

Fig. 3. The epidemic of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-). The graph shows the number of *Salmonella* isolates from human infections at the French National Reference Centre for *Salmonella* during 2000 to 2016. The blue bars depict the total number of *Salmonella* spp. isolated by year over the defined period; the red plot depicts the number of *Salmonella* Typhimurium (1,4,[5],12:i:-) isolated by year.

exposure to STIs alongside therapeutic antimicrobials, created the "perfect storm" for the emergence of this specific MSM-associated AMR lineage.

Foodborne dissemination of antimicrobial resistance

Humans are exposed to animal sources of AMR genes and bacteria through the food chain. The need for a "one-health" (i.e., considering the span of humans, animals, and their environment) strategy for AMR and infectious disease for surveillance and containment across the different sectors is well recognized. Nontyphoidal *Salmonella* (NTS), which is among the most common pathogens of humans and animals, are key for understanding AMR dynamics from a one-health perspective. In 2004, the Infectious Disease Society of America (IDSA) issued a report that presented a plausible catastrophic scenario of a highly fatal epidemic of MDR-NTS, illustrating how virulent AMR strains could rapidly escalate into major foodborne outbreaks threatening our food security. Indeed, large foodborne NTS outbreaks have been observed in recent decades, and NTS exhibiting resistance to last-line antimicrobials are beginning to be isolated.

The continued occurrence of MDR *Salmonella* Typhimurium (one of the most common types of NTS) as a cause of human infection personifies the one-health aspect of AMR and also highlights repeating patterns of AMR evolution. Antimicrobials have been used to treat and prevent infections in livestock since their discovery but were also used as growth promoters from the 1950s. In the early part of the 1960s, an increasing number of *S.* Typhimurium with transferable MDR phenotypes began to be isolated in the United Kingdom, with the first outbreak of MDR *S.* Typhimurium (phage type 27) in humans reported in 1959. This outbreak affected 102 patients; ~5% of isolates were resistant to streptomycin, sulfonamides, and tetracycline (*29*). In 1963, *S.* Typhimurium phage type DT29 emerged in the United Kingdom following the adoption of intensive farming methods using antibiotics for the rearing of calves (*30*). Subsequently, in 1965, >1200 and >500 MDR *S.* Typhimurium were isolated from cattle and humans, respectively. A recent WGS NTS investigation revealed that the AMR gene cassettes present in these early U.K. *Salmonella* outbreaks differed from those in historical *Salmonella* outbreaks in France, despite geographic proximity (*31*). This observation suggests that the emergence of MDR *S.* Typhimurium was caused by the independent acquisition of multiple AMR determinants followed by country-specific clonal expansions.

> ***"...globalization of the food industry means that inappropriate antimicrobial use in one part of the world has implications even for countries with strong controls on their own usage."***

Observations from the 1960s were repeated in the 1980s when *S.* Typhimurium phage type DT104 with a genomic island encoding resistance against ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines emerged in U.K. cattle (*32*). This epidemic strain successively acquired resistance to quinolones and trimethoprim. Over the coming years, DT104 became widely distributed in cattle, poultry, pigs, and sheep and in 1996, >4000 human infections were associated with MDR DT104 in the United Kingdom. MDR DT104 spread internationally throughout the 1990s, particularly in continental Europe and North America, and became established in multiple domestic animal populations. By 2001, DT104 represented >50% of all *S.* Typhimurium isolates in Eastern Europe (*33*). Local and global transmission routes were reconstructed by WGS, and the role of this zoonotic pathogen in the spread of AMR through interspecies transmission was elucidated (*34*). These data may have cast doubt on the dominance of local animals in spreading MDR DT104 to humans, but importantly, they highlighted substantial gaps in our AMR surveillance. Notably, the general contribution of imported food in spreading AMR bacteria to humans remains poorly understood.

The latest foodborne *S.* Typhimurium epidemic was associated with swine and attributed to a monophasic variant (1,4,[5],12:i:-), which emerged in Europe in the mid-2000s, as highlighted by spread of the clone in France from 2008 (Fig. 3) (*35*). Sequence data identified these organisms as one clone, despite belonging to multiple phage types, that was distinct from monophasic *S.* Typhimurium previously described in Spain and North America. These were found to have become MDR through the acquisition of a composite transposon, which replaced the flagella operon. These isolates had also acquired a genomic island, which encoded resistance to several heavy metals in pig-feed supplements. This European monophasic variant has now been reported in swine in the Midwestern United States, where it has become resistant to quinolones and third-generation cephalosporins (*36*).

It was proposed relatively early on that use of penicillins and tetracyclines in livestock was responsible for the emergence of MDR *S.* Typhimurium. This hypothesis was controversial, owing to the complexities of NTS epidemiology and the lack of molecular tools allowing high-resolution tracking of the incriminated bacteria in the different ecosystems. In the 1980s, epidemiology, combined with early molecular typing techniques, concluded that most AMR variants of NTS in the United States could be traced to animals (*37*). Antimicrobial use for growth promotion was banned by the European Union in 2006 and heavily regulated in the United States in 2017. However, globalization of the food industry means that inappropriate antimicrobial use in one part of the world has implications even for countries with strong controls on their own usage. The recent example of the worldwide dissemination of MDR *Salmonella* Kentucky ST198 via African poultry further highlights the requirement for global one-health approaches to tackle AMR (*38*).

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Staying one step ahead

It is indisputable that efforts to kill bacteria with chemicals will result in the selection, propagation, and dissemination of resistant variants. Data generated through WGS have revealed the rapid pace at which the bacteria can adapt to these chemicals. It is evident that some AMR pathogens have outpaced us, with untreatable infections appearing in hospitals and the community; but WGS studies have provided us with the tools and knowledge to initiate an intelligence-driven fightback. In particular, population genomics data at various spatiotemporal scales highlight many repeating patterns in the emergence and spread of AMR. The predictability of these evolutionary processes offers the opportunity to develop strategies to minimize the chance that new AMR clones are generated during individual treatment that will spread locally. For example, combination and sequential therapies may create conditions that constrain the fitness of emerging resistant mutants (*39*). These strategies are based broadly on the principle that adaptation to one class of antimicrobial drug may incur collateral sensitivity to another, such that their coordinated use imposes a roadblock to the emergence and spread of resistance. As diagnostics are generally lacking, the most practical option is likely to be empirical antimicrobial rotation as opposed to patient-tailored therapies. In theory, antimicrobial combinations or cycling can be employed at different levels (e.g., patients, wards, hospitals) and time scales (e.g., hours, days, months), depending on whether the goal is to limit the emergence of AMR within patients, or to confine the transmission of AMR variants. However, much work is required to determine the most effective way to restrict emergence and spread of differing resistance phenotypes in different settings (*40*). These approaches have the potential to lengthen the life of current antimicrobials and are vital for sustaining the efficacy of new antimicrobials as they are introduced.

A further important insight from WGS is that while resistance arises constantly during individual infections, most AMR variants represent a minimal risk with limited potential for transmission beyond the index patient. Hence, the major burden of AMR is associated with a few high-risk clones that spread easily and accumulate additional AMR phenotypes. It is these clones that represent the greatest risk beyond the individual patient and should be targeted more aggressively for containment. Work is still needed to understand the mechanisms underlying these apparently superfit AMR clones, and WGS studies will be vital for this process. Even in the absence of precise mechanisms, WGS can be deployed immediately for hospital infection control and public health surveillance to identify and target clones with epidemic potential as they arise.

The spatiotemporal dynamics of AMR evolution revealed by WGS studies clearly illustrate that microbial populations do not respect political boundaries; hence, it is imperative that AMR genomic surveillance data are combined internationally between different sectors in a one-health approach (i.e., across medical, veterinary, agricultural, and environmental settings). Such data sharing is essential to harness the power of genomic surveillance to identify and monitor evolutionary trends and population dynamics and to identify superfit AMR clones as they emerge and spread. The rapid pace of the global spread of AMR organisms, such as fluoroquinolone-resistant *Shigella* (*22*), indicates that these efforts have to be implemented in real time, as has been argued for the emergence of novel pathogens (*5*). This is the vision of the Global Microbial Identifier Project, the WHO, and other international bodies, but it has yet to gain international support from governments and industries.

AMR is a truly global health problem, one that we cannot ignore or attempt to counter with increasingly powerful antimicrobial agents. WGS has allowed us to understand the dynamics of AMR and the chaos we have created through haphazard antimicrobial usage. The data are stark. However, recognizing the complexity and assessing the magnitude of the task ahead is the first fundamental step in tackling the global AMR crisis. We are now at a pivotal point, and what happens next is likely to dictate the future of infectious disease control. Genomics has outlined several repeating patterns in the emergence and spread of AMR bacteria, and although we cannot stop bacterial evolution, we can try to channel it. Through coordinated efforts, intelligent surveillance, and a more fundamental understanding of AMR mechanisms, we can learn to use antimicrobials more effectively and extend their longevity.

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REVIEW

Worldwide emergence of resistance to antifungal drugs challenges human health and food security

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The recent rate of emergence of pathogenic fungi that are resistant to the limited number of commonly used antifungal agents is unprecedented. The azoles, for example, are used not only for human and animal health care and crop protection but also in antifouling coatings and timber preservation. The ubiquity and multiple uses of azoles have hastened the independent evolution of resistance in many environments. One consequence is an increasing risk in human health care from naturally occurring opportunistic fungal pathogens that have acquired resistance to this broad class of chemicals. To avoid a global collapse in our ability to control fungal infections and to avoid critical failures in medicine and food security, we must improve our stewardship of extant chemicals, promote new antifungal discovery, and leverage emerging technologies for alternative solutions.

The rapid emergence of multidrug-resistant pathogenic fungi and the better-publicized threat of antibiotic-resistant bacteria together pose a considerable threat to disease control across diverse anthropogenic systems. These microbes respond adroitly to human-induced natural selection through chemical treatments and nimbly hijack human globalization pathways (*1*), thus disseminating the problems worldwide. Today, crop-destroying fungi account for perennial yield losses of ~20% worldwide, with a further 10% loss postharvest. Fungal effects on human health are currently spiraling, and the global mortality rate for fungal diseases now exceeds that for malaria or breast cancer and is comparable to those for tuberculosis and HIV (*2*). Fungal infections have hitherto been greatly neglected relative to other classes of infectious disease, despite their ubiquity.

The first antifungal chemicals used in human health care, nystatin and the polyenes, were discovered in the 1950s, and copper and sulfur fungicides were first used to control crop disease more than 150 years ago. Today, systemic antifungals and fungicides are used as frontline treatments for fungal diseases in humans and plants. Fungal pathogen control can, however, be ephemeral because of the rapid development of resistance to the chemicals. Fungi have highly plastic genomes and reproduce rapidly. The combination of these properties quickly generates variants selected for resistance. For plant pathogens, the pace of breakdown of antifungal protection is enhanced by monoculture cropping practices, as large swathes of genetically uniform crops provide ideal breeding and feeding grounds for the rapid emergence of fungicide-resistant variants. In humans, long periods of prophylactic treatment in at-risk patients can similarly lead to the emergence of antifungal resistance (*3*). Resistance of clinical pathogens to all licensed systemic antifungals has been documented, although the rate of emergence varies among drug classes (Fig. 1) (*3*). Likewise, despite the wider range of fungicides licensed for use in agriculture, resistance to each main class of fungicides has emerged in some major pathogens (Fig. 1). This threat is exacerbated by the additional threat of withdrawal of some chemical classes because of regulatory changes in jurisdictions such as the European Union (EU).

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Antifungals for the treatment of fungal diseases in the clinic and the field

The chemical control of fungal pathogens that cause diseases in animals and crops has progressed from the use of inorganic chemicals to the use of organic surface protectant chemicals and then to the use of systemically acting fungicides. Approximately nine times more antifungal compounds are available to control crop diseases than to treat systemic animal infections. Licensed treatments for humans are limited to four frontline classes of drugs (Fig. 1): The polyenes (such as amphotericin B) disrupt the structure of cell membranes by sequestering the fungal membrane sterol ergosterol. The pyrimidine analog 5-fluorocytosine (5-FC) blocks pyrimidine metabolism and DNA synthesis. The newest class of antifungals, the echinocandins, inhibits (1-3)-β-D-glucan synthase and disrupts cell wall biosynthesis. The fourth and most widely used class of fungicides, the azoles, blocks ergosterol biosynthesis through inhibition of lanosterol 14-α-demethylase. Most fungicides for crop disease target mitochondrial function, the cytoskeleton, or ergosterol biosynthesis (Fig. 1), although some specialized chemicals, such as the azanaphthalenes for powdery mildew control, target other pathways. However, the azoles remain the dominant chemicals in the treatment of fungal infections in crops, humans, and livestock, with five licensed clinical azole antifungals and 31 available for crop protection.

Parallel drivers of fungicide resistance in the clinic and the field

Human population growth, urbanization, and economic prosperity have fueled demands for increasing quantities and varieties of food. Intensive agriculture has too often responded to this demand with crops bred for maximum productivity under the protection of broad-scale pesticide applications, inadvertently breeding out the plants' own defenses. In parallel, the number of humans at risk from fungal infections is rising rapidly with increases in populations that are particularly susceptible because of age, medical interventions, or HIV infection. Medical advances resulting in greater initial survival rates for patients with cancer or organ transplantation can leave these patients susceptible to secondary attacks from opportunistic fungi, leading to increasing use of antifungal drugs in clinical practice (Fig. 2 and table S1).

The global movement of people and global trade in produce have hastened the free flow of fungal pathogens from country to country, bringing pathogens into contact with naïve hosts (*1*) (Fig. 3). In the clinical setting, new species of multidrug-resistant pathogenic fungi are emerging. *Candida auris,* first described in Japan in 2009 after isolation from a patient's ear, is responsible for rapidly increasing hospital-acquired invasive infections worldwide (*4*). This fungus is now resistant to all clinical antifungals (*5*) and presents a threat to intensive care units because it can survive normal decontamination protocols (*6*). The emergence of resistance in *Candida glabrata* has coincided with this species becoming the predominant bloodstream pathogen recovered from patients, largely because of the increasing prophylactic use of echinocandins and azoles (*7*). There is also a growing threat from filamentous pathogenic fungi that are intrinsically resistant to a broad range of antifungals, such as *Aspergillus terreus* (*8*), *Scedosporium* spp. (*9*), *Fusarium* spp. (*10*), and members of the Mucorales (*11*).

Simultaneously, we are witnessing the continual emergence of new races of plant-infecting fungi able to overcome both host defenses and chemical treatments (*12*), as well as the evolution of these traits in existing major pathogens (*13*, *14*). The first case of resistance against the benzimidazoles (MBCs) was reported in 1969 (*15*), and now MBC resistance is known to occur in more than 90 plant pathogens (*16*). Azole resistance in a plant pathogen was first reported in 1981 (*17*), but azole resistance is generally partial, in contrast to the complete control failures seen for MBCs (*18*). Resistance to strobilurins (QoIs) was reported in field trials even before commercial introduction and in wider field

populations within 2 years of release (*19*). A new generation of succinate dehydrogenase inhibitors (SDHIs) was introduced in 2007, but by 2017 resistant field isolates were found in 17 pathogen species (*20*). Pathogens with resistance to MBCs, azoles, QoIs, and SDHIs include the major wheat pathogen *Zymoseptoria tritici*, banana black sigatoka pathogen *Mycosphaerella fijiensis*, cereal powdery mildew fungus *Blumeria graminis*, the emerging barley pathogen *Ramularia collo-cygni*, and the apple scab fungus *Venturia inaequalis*. For *Botrytis cinerea* (a generalist pathogen that causes gray mold, particularly on soft fruits), resistance against 15 different classes of systemic and protectant fungicides has been reported (*21*).

Parallel evolution of resistance mechanisms in the clinic and the field

The selective pressure exerted on fungi by single-site–inhibiting fungicides has resulted in similar adaptations arising over time in disparate fungal species. Parallel evolution of resistance extends across clinical and plant-pathogenic fungi, with the same key resistance mechanisms occurring independently in both.

Mutations resulting in conformational changes to the drug target site are the most common form of resistance in pathogenic fungi. Target-site mutations have been reported in candin-resistant clinical pathogens and MBC-, QoI-, and SDHI-resistant plant pathogens, as well as azole-resistant strains in agricultural and clinical settings. A single mutation, Gly^{143}→Ala in cytochrome *b*, has emerged in the field in more than 20 species under selection by QoIs (*14*). Moreover, the Tyr^{137}→Phe substitution in CYP51 (P450 cytochrome) has been found in multiple plant pathogens with partial azole resistance, and Tyr^{132}→Phe also occurs at the equivalent residue in *Candida albicans* (*18*). Promoter changes resulting in up-regulation of the fungicide target are also common across clinical and plant-pathogenic fungi (*22*). In *Aspergillus fumigatus*, tandem repeats in the CYP51A promoter region occur together with downstream single-nucleotide polymorphisms (SNPs) in the coding region, conferring a multi-azole resistance phenotype (*23*).

A third resistance mechanism involves reducing intracellular drug accumulation by up-regulation of efflux pumps, such as adenosine triphosphate–binding cassette transporters or major facilitators. Their up-regulation may result from promoter insertions or transcription factor gain-of-function mutations (*3*, *24*).

Further resistance mechanisms have been identified in clinical pathogens. Activation of stress response pathways by Hsp90 can unleash cryptic diversity, potentiating the evolution of resistance to azoles, echinocandins, and polyenes in *Candida* and *Aspergillus* species (*25*). Structural genomic plasticity can result in resistance, with chromosome arm duplications leading to efflux pump and target-site overexpression in *C. albicans* (*24*, *26*). Hypermutator strains of *C. glabrata* and *Cryptococcus neoformans*, with the potential to evolve rapidly in response to host and drug selection, were recently reported (*27*, *28*).

Dual use of azoles in the clinic and the field

The azoles are the most widely deployed class of fungicides in crop protection, totaling in excess of 26% of all fungicides across the EU (*29*). Azoles are also frontline drugs used in humans and animals; however, such multiple use seems to have promoted azole resistance in an opportunistic pathogen of humans (*29*, *30*), the saprotroph *A. fumigatus*. This species colonizes decaying vegetation in fields, forests, and compost heaps but is also capable of invading immunocompromised humans. Multi-azole–resistant *A. fumigatus* has been recovered from environmental and clinical samples globally. In the Netherlands, more than 25% of clinical *Aspergillus* strains carry azole resistance alleles (*31*). Azoles are increasingly failing as frontline therapies, with associated patient mortality approaching 100% (*31*). Population genomic analyses have shown that azole-resistant alleles in *A. fumigatus*

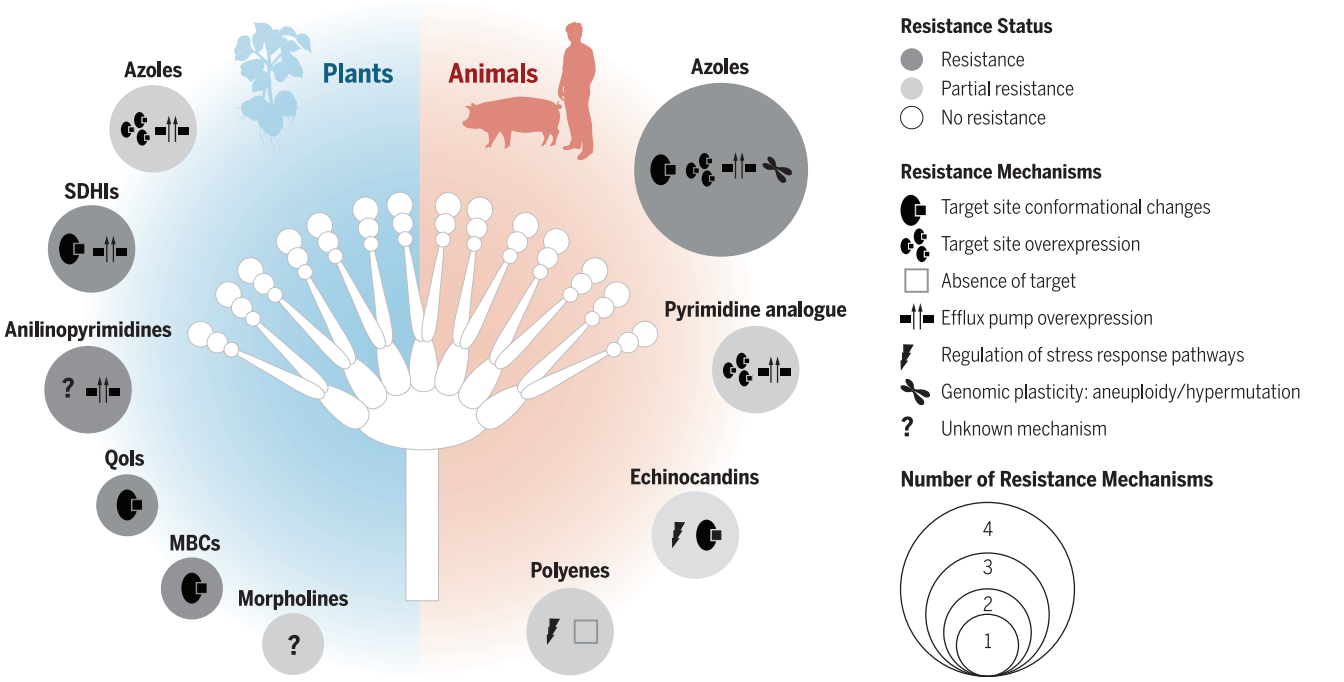

ILLUSTRATION: CHARLOTTE GURR, ADAPTED BY K. KHOLOSKI

Fig. 1. Current classes of drugs used against plant and animal fungal infections and known mechanisms of resistance to them. The six main classes of fungicides are the morpholines, which inhibit two target sites within the ergosterol biosynthetic pathway, Δ14-reductase and Δ8-Δ7-isomerase (this reduces the risk of target-site resistance, but their intrinsic antifungal activity spectrum is narrower than those of other antifungals); the azoles (used also in animal infections), which target the ergosterol biosynthetic pathway; the benzimidazoles (MBCs), which interfere with the cytoskeleton by binding to β-tubulin, thus preventing the assembly of microtubules; the strobilurins (QoIs) and succinate dehydrogenase inhibitors (SDHIs), which both inhibit the electron transfer chain of mitochondrial respiration, with the SDHIs inhibiting complex II (succinate dehydrogenase) and the QoIs inhibiting complex III (the quinone outside binding pocket of cytochrome *b*); and the anilinopyrimidines, which may target mitochondrial signaling pathways. Three other antifungal classes are used for animal fungal infections: the echinocandins, which inhibit cell wall biosynthesis; the pyrimidine analogs, which interfere with nucleic acid biosynthesis; and the polyenes, which bind ergosterol.

are associated with selective sweeps when azole use is high, as in India (*32*). Moreover, recombination in *A. fumigatus* generates new combinations of azole resistance alleles (*32*). Investigations are now under way to assess the relative contributions of clinical and environmental selection to azole resistance in *A. fumigatus* and to identify the most problematic environmental applications of azoles. The potential conflict between the level of agricultural use and the durability of clinical effectiveness of azoles highlights how limited the antifungal toolbox is, where neither "side" can afford to lose a mode of action (*33*).

Most cases of fungicide and antifungal resistance across field and clinic settings appear to have arisen by the repeated independent evolution of resistance to successive fungicides within numerous fungal species. This is where evolution of antifungal resistance differs fundamentally from that of antibacterial resistance, which is frequently transferred between pathogens of animals and humans via the "mobilome" of plasmids and phage (*34*). Some evidence indicates horizontal gene transfer among fungi (*35*), but this fungal gene transfer occurs over longer time scales than gene transfer among bacteria and the dynamics of resistance arising by this route is thus far negligible.

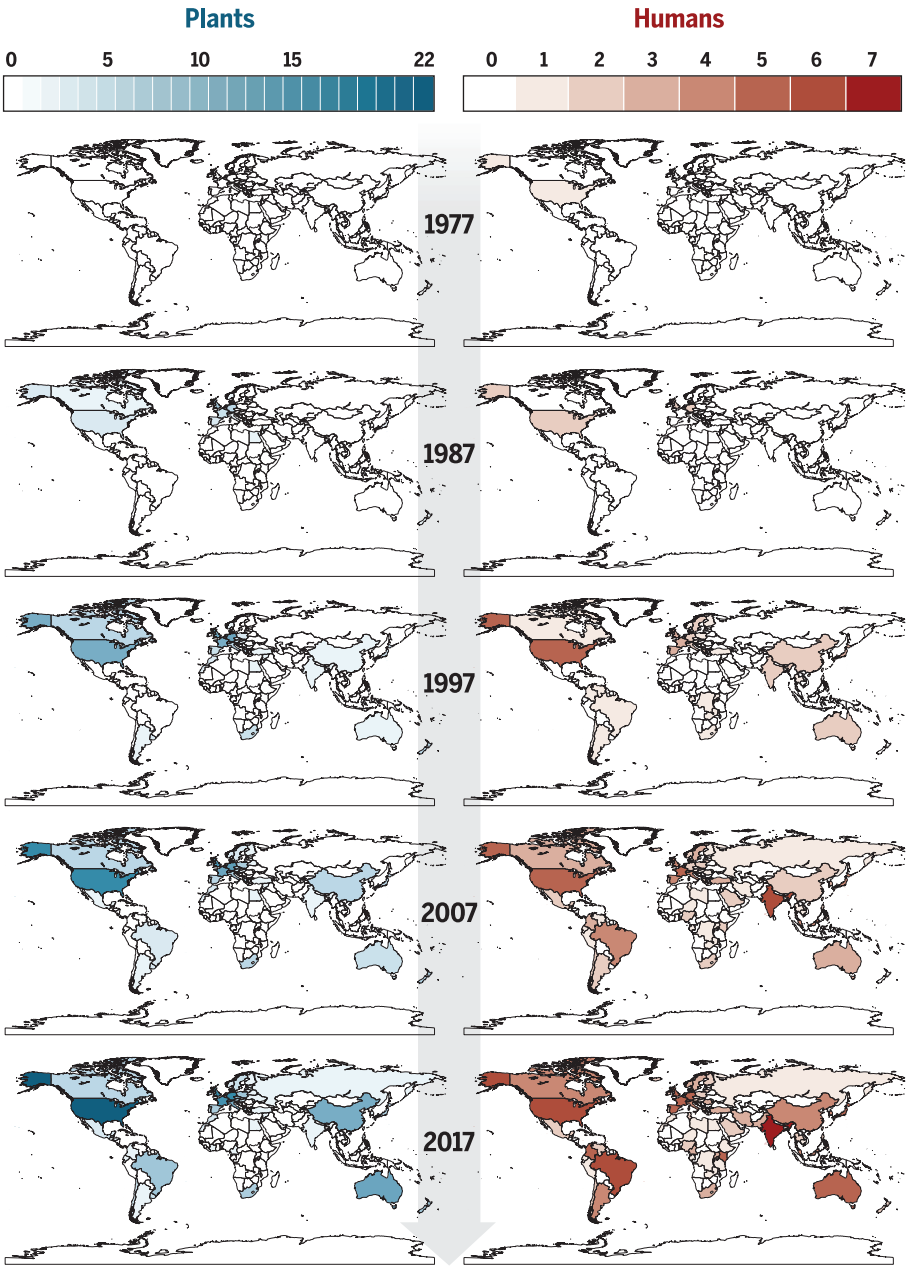

ILLUSTRATION: CHARLOTTE GURR. ADAPTED BY K. KHOLOSKI

Fig. 2. Fungal species with reported antifungal resistance, by country. Increasing color intensity reflects a growing number of reports. The plant maps depict spatiotemporal records of resistance of crop pathogens to azoles (blue scale). The human maps depict spatiotemporal records of resistance of the pathogens *A. fumigatus*, *C. albicans*, *C. auris*, *C. glabrata*, *Cryptococcus gattii*, and *Cryptococcus neoformans* to azoles (red scale). The data are derived from peer-reviewed publications as of March 2018, reporting the occurrence of cases of resistance up to 2017 (the list of publications is available in table S1).

Prospects for diversifying the toolbox for fungal control

To counter the escalating risks of fungal disease, we need to discover antifungal chemicals with new modes of action, hinder the emergence of resistance in extant chemicals by better stewardship, and develop new disease control strategies to avoid overreliance on fungicides.

Development of new antifungals

The rate of emergence of fungicide resistance (Fig. 2) is greater than the pace of fungicide discovery, and the long registration process for new compounds adds further delays. This situation parallels the situation for antibiotics. Increased research activity is thus needed to develop new antifungal drugs (*36*). Recently, substantial progress has occurred in this field, with at least 11 antifungals in phase 1 and 2 clinical trials and at least two in the agricultural chemicals pipeline. Several of these are derivatives of commonly used antifungal chemicals, such as ergosterol biosynthesis and cell wall biosynthesis inhibitors, engineered for higher efficacy, and others have new modes of action. Combining molecular modeling, combinatorial chemistry, and high-throughput screening has the potential to develop chemicals with reduced resistance risk (*37*).

Stewardship of existing compounds

Robust global strategies are needed to slow the development of antifungal resistance. Combining different modes of action, either in mixtures or in alternating treatments, may slow the emergence of resistance. For example, combinations of fluconazole, flucytosine, and amphotericin B can effectively treat HIV-associated cryptococcal meningitis (*38*). In agriculture, mixtures of fungicides with different modes of action are already widely recommended (*39*), with some formulations available only as mixed products. Where target-site mutations confer high levels of resistance, lower doses of antifungals should be favored (*40*, *41*). However, this results in a trade-off between the immediate gain of treatment effectiveness and the longer benefit from slowing the selection of resistance. Improvements in molecular diagnostics are also needed, both for the identification of fungal pathogens so that antifungals can be used appropriately and for the detection of specific resistance alleles, as the monitoring of resistance is a vital part of stewardship (*42*).

Integrated disease management

To reduce our reliance on chemical control alone, we must develop more nonchemical control measures to use where effective fungicides are no longer available or to use in combination with fungicides to reduce the selective pressure

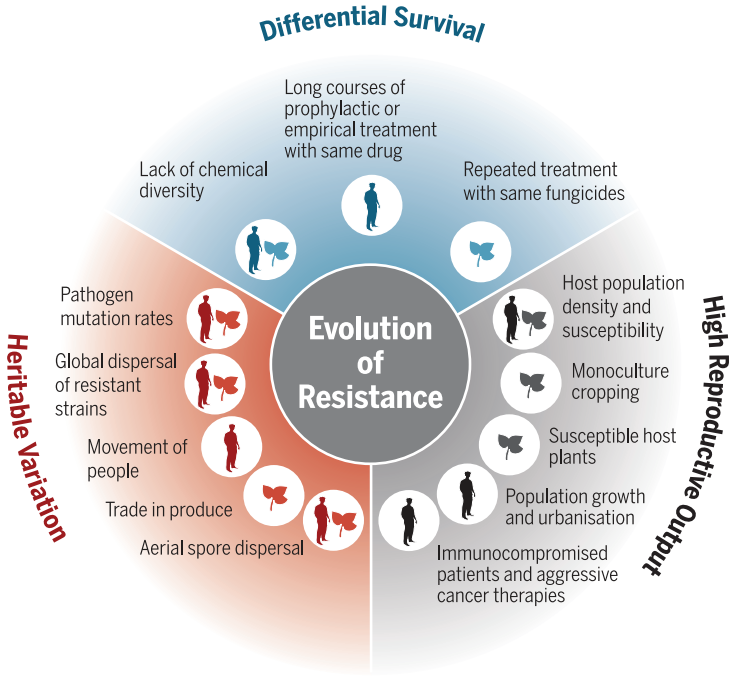

Fig. 3. Evolutionary drivers of antifungal resistance: heritable variation, high reproductive output, and differential survival.

on each component. In crops, the development of innate disease resistance through the selection of major pathogen-resistance alleles is widely used to breed disease-resistant cultivars. However, this approach is slow, with a 20-year lag from finding a suitable disease-resistance gene to releasing it in commercial lines. Marker-assisted breeding can speed up the recombination of multiple disease-resistance alleles, but it still takes approximately a decade (*43*). Transgene cloning, or gene editing, is faster still (requiring ~2 years), but no crops with transgenic antifungal disease resistance have yet been released commercially. The high degree of specificity between host and pathogen for major resistance genes (*44*) means that pathogens can also rapidly evolve to overcome this strategy. However, "evolution-smart" disease-resistant crops with pyramided pathogen-resistance genes or mosaic deployment of resistant varieties may provide greater durability of disease control. Minor resistance genes, such as those for the antifungal chitinases and glucanases, carry the advantage of broad-spectrum activity (*45*) but introduce the possible disadvantage of yield penalties, as well as providing incomplete protection. Further sources of genetic disease resistance can be found in the gene pools of crops' wild relatives, which may be introduced into modern crop varieties through introgression or transgenesis (*43*).

In humans, advances in combination antiretroviral therapy to halt HIV-AIDS progression, gene therapies under development for cystic fibrosis, and tissue engineering for rejection-free transplantation can reduce vulnerability to fungal infections in the corresponding patient cohorts. Also, the first antifungal vaccine against *C. albicans* is undergoing clinical trials (*46*), and the use of bioengineered T cells to augment host immunity is being explored (*47*). Lastly, the identification of human genetic biomarkers associated with susceptibility to fungal diseases, such as SNPs in the immune mediator *PTX3* (*48*), provides a new path to identify patient groups in which antifungal treatments could be reduced.

The rapidly growing fields of synthetic biology and epigenomics are now converging to develop antifungal treatments on the basis of RNA interference (RNAi). Bidirectional cross-kingdom microRNA (miRNA) trafficking between plants and fungi is being developed to fight pathogens (*49*) such as *B. cinerea*, which uses miRNA virulence effectors to silence host plant immune genes (*50*, *51*). Current research avenues include identifying new targets for RNAi and, crucially, developing systems for the stable and targeted delivery of RNA silencing through genetic engineering of the host plant or exogenous application of synthetic RNA (*50*–*52*). Although such approaches have not yet been used to treat fungal infections in the clinic, the discovery of RNAi as a promising clinical antifungal strategy is potentially transformational.

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SUPPLEMENTARY MATERIALS

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Table S1
References (*53*–*323*)

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ILLUSTRATION: CHARLOTTE GURR. ADAPTED BY K. KHOLOSKI

REVIEW

Prospects for harnessing biocide resistance for bioremediation and detoxification

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Prokaryotes in natural environments respond rapidly to high concentrations of chemicals and physical stresses. Exposure to anthropogenic toxic substances—such as oil, chlorinated solvents, or antibiotics—favors the evolution of resistant phenotypes, some of which can use contaminants as an exclusive carbon source or as electron donors and acceptors. Microorganisms similarly adapt to extreme pH, metal, or osmotic stress. The metabolic plasticity of prokaryotes can thus be harnessed for bioremediation and can be exploited in a variety of ways, ranging from stimulated natural attenuation to bioaugmentation and from wastewater treatment to habitat restoration.

Microorganisms in pristine ecosystems as well as those in anthropogenically disturbed habitats are constantly challenged by combinations of chemicals and physical stresses. Natural habitats can experience combinations of conditions from high salinity and osmolarity, desiccation, ultraviolet radiation, high pressure, or extremes of pH or temperature (*1*). Industrial, agricultural, and domestic activities lead to the release of organic and inorganic compounds toxic to a wide range of organisms in the environment. Microbes exposed to such conditions can rapidly develop physiological and/or genetic adaptations to resist environmental constraints. Harnessing the metabolic capacities of prokaryotes and their adaptive potential is of interest for a broad range of applications for environmental clean-up as well as for treatment of domestic and industrial waste.

Microbial tolerance and resistance mechanisms

The mechanisms that enable bacteria to survive typical environmental stressors, such as toxic concentrations of organic pollutants and changes in temperature or osmolarity, are well understood (*2*–*4*). Preventing damage to the cell envelope and cellular membranes are pivotal for prokaryote survival (*5*). Hence, one of the first responses to toxic assault is membrane repair to reestablish membrane fluidity and rigidity. In Gram-negative bacteria, this occurs with the insertion of saturated and *trans*-configurated unsaturated fatty acids, whereas in Gram-positive bacteria, *iso*-branched fatty acids are inserted (*6*). Cell-surface properties can also be modified during exposure to stressors by the release of outer-membrane vesicles, which increase surface hydrophobicity. This phenomenon can stimulate biofilm formation, making bacteria yet more tolerant to environmental stressors (*7*). Bacteria can also change their morphology in the presence of toxic concentrations of organic pollutants, increasing their overall size and decreasing surface-to-volume ratio (*5*).

Many bacteria respond to stresses by inducing synthesis of specific membrane efflux pumps. This response is well understood in bacteria capable of withstanding high concentrations of organic solvents such as benzene, toluene, ethylbenzene, and xylene (BTEX). BTEX are excreted from membranes by energy-driven protein pumps belonging to the root nodulation (RND) family of membrane proteins. RND proteins are known in other bacteria to transport antibiotics and contribute to multidrug resistance (*3*). Cross-protection to different stresses is common. For example, bacterial cells that adapt to a given solvent also show increased tolerance to other solvents, heavy metals, antibiotics, and several forms of physical-chemical stress. Because bacterial adaptive physiological responses are inducible, it is therefore possible to pre-adapt the cells for potential applications at contaminated sites (*5*).

Role of environments in tolerance and resistance selection

Although any environment ultimately selects for the survival and proliferation of specific microbial genotypes, extreme and polluted environments showcase the power of such selective forces. Polluted environments are frequently characterized by high concentrations of toxic substances that can appear in sudden, infrequent, but ephemeral bursts such as oil spills (*8*), but equally, chronic pollution can arise from long-term input of pollutants (*9*). An influx of high concentrations of toxic compounds can lead to dramatic shifts in microbial community composition and diversity (Fig. 1, top) (*10*). Consequently, carbon and nutrients in the system that are no longer used by sensitive phenotypes can be used for growth by resistant phenotypes (Fig. 1, top) (*11*). Additionally, polluting compounds can become an exclusive source of assimilable nutrients or electron donors or acceptors for resistant microorganisms (Fig. 1, bottom) (*11*). For example, oil-degrading bacteria occur at typically low abundances in marine environments but respond with astonishingly rapid blooms during oil spills (*12*). Even for synthetic chemicals considered to be xenobiotic—such as chlorinated solvents, pesticides, and the plastic poly(ethylene terephthalate)—release into the environment, and long-term pollution selects for the appearance and proliferation of mutants with naturally recombined metabolic pathways, which profit from the exclusivity of the toxic compound for growth (*13*–*15*). Natural recombination is largely the result of abundant horizontal gene flow in prokaryote communities. Diverse mechanisms have been implicated in gene flow, such as plasmid conjugation, natural transformation, and integrative and conjugative or transposable elements (*11*). Extreme toxicity resistance as a result of RND-type efflux mechanisms may thus be a prerequisite for further adaptation by keeping the intracellular concentration of the toxicant low enough to permit its metabolism (*16*).

As worldwide environmental concerns shift from high contamination loads of legacy chemicals—such as oil, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls—toward low concentrations of biologically very active molecules—including antibiotics, other pharmaceuticals, and ingredients of household and consumer care products—the question is what types of microbial resistance will be selected by low and chronic concentrations of these chemicals. Although low concentrations of chemicals can be toxic to some lineages and may result in selection of resistant phenotypes, as the widespread emergence of antibiotic resistances attests, the distinct proliferation of "compound-degrader" phenotypes may be more difficult to discern. Conceivably, micropollutant degraders might have more advantage in oligotrophic environments (*17*), where available nutrients are scarce and the ability to metabolize micropollutants may be particularly competitive.

Concepts for harnessing toxicant-tolerant or -resistant bacteria

An important outcome of adaptation and selection in contaminated environments is that sites chronically polluted with organic compounds naturally restore over time and diminish the pollution load (*18*). Such natural attenuation and restoration processes may, however, take decades (*19*). Nevertheless, they require little technical intervention or cost. The spontaneous adaptation and selection that has led to the appearance of (naturally recombinant) bacteria capable of resisting or degrading contaminants has since long attracted interest for potential applications

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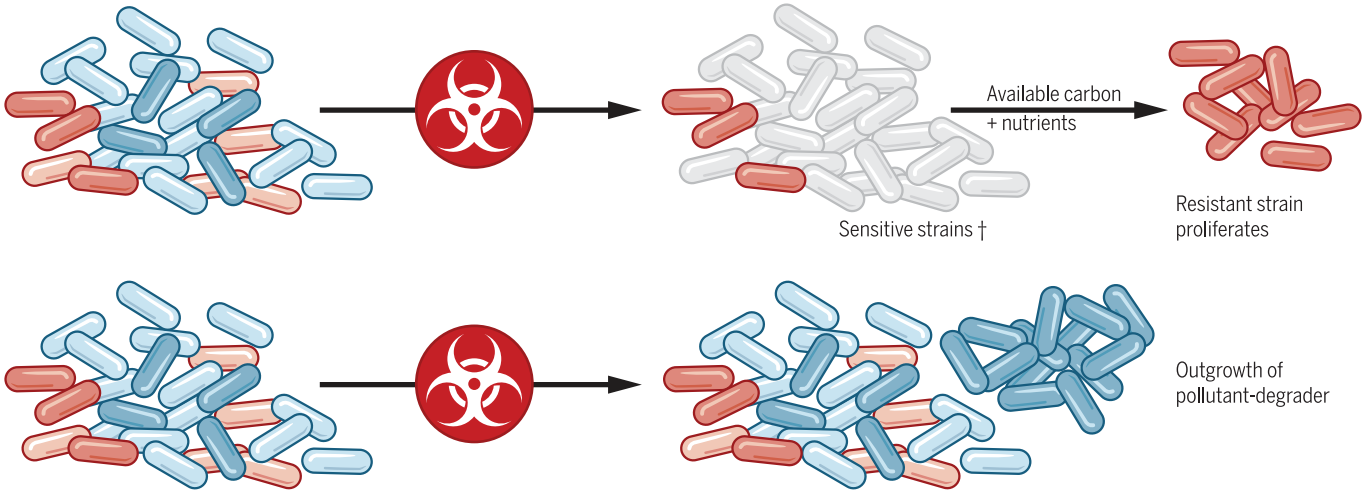

Fig. 1. Environmental selection of adaptive phenotypes to toxic compound stresses. (**Top**) Exposure of a diverse bacterial community to toxic concentrations of chemicals inhibits or kills sensitive individuals. Resistant organisms profit from the availability of unused carbon and nutrients in the system to proliferate. (**Bottom**) Toxic organic compounds themselves can be used as an exclusive growth substrate for low numbers of preexisting specialist bacteria in the community or for newly arising mutants. These lineages will proliferate by consuming the toxic compound, potentially leading to the spontaneous natural attenuation of a contaminated site. Specialist degrader bacteria may additionally profit from toxicity-resistance mechanisms.

elsewhere. The enrichment or isolation of promising pollutant-degrading bacteria, growth under laboratory conditions, and formulation for use in similar conditions and context—a process called bioaugmentation—could potentially shorten the long on-site adaptation process and accelerate remediation.

Bioaugmentation has been successfully applied at sites contaminated with organohalogen compounds. Organohalide-respiring bacteria (OHRB) such as *Dehalococcoides mccartyi*, *Dehalogenimonas* spp., and *Dehalobacter* spp. use chlorinated solvents and/or pesticides as their sole terminal electron acceptors for growth (*20*). Organohalide respiration is probably evolutionarily ancient (*21*), but traces of recent or even ongoing genetic adaptation are detectable in the genomes of these species. Precultured stocks of microbial consortia containing OHRB have been successfully applied so as to improve bioremediation of sites contaminated with chlorinated solvents such as tetrachloroethene (Fig. 2) (*20*, *22*). OHRB augmentation has been shown to be essential for on-site chlorinated solvent bioremediation because stimulation of the autochthonous OHRB frequently leads to accumulation of a more toxic transformation product, vinyl chloride (*23*).

Widespread pollution with hexachlorocyclohexanes (HCHs) arose around the world during production of the currently largely banned pesticide, the γ-HCH isomer lindane. Bacteria adapted to using HCHs as their sole carbon and energy sources have been discovered at HCH-contaminated sites (*24*) as a result of natural recruitment and recombination of existing genes and subsequent mutations. Such bacteria have been isolated, cultured in larger quantities, specifically formulated, and successfully used in the bioaugmentation of HCH-contaminated areas (*25*).

Oil bioremediation

Crude oil is toxic to metazoan life yet is a supply of extremely energy-rich carbon sources for hydrocarbonoclastic bacteria. Hydrocarbonoclastic bacteria are ubiquitous and evolutionarily old lineages that have adapted to oil components released at natural oil seeps (*26*, *27*). Typically, their population size in the absence of oil spills is very small, but they bloom during oil contamination. For example, *Oceanospirillales* spp. can compose 90% of the local marine bacterial community after oil spillage (*27*). Two well-known species, *Alcanivorax borkumensis* and *Oleispira antarctica*, have evolved several adaptive strategies to optimize access to their poorly water-soluble aliphatic hydrocarbon substrates (*27*, *28*). These include an increase in cell surface hydrophobicity that is thought to favor partitioning of substrates into the cell envelope, as well as production of biosurfactants to increase the ambient solubility of the aliphatic hydrocarbons. Interestingly, *A. borkumensis* is also able to directly incorporate fatty acids, resulting from oxidation of aliphatic hydrocarbons, into its cell membrane (*28*).

> ***"Although any environment ultimately selects for the survival and proliferation of specific microbial genotypes, extreme and polluted environments showcase the power of such selective forces."***

Although bioaugmentation of oil spills is often revisited, the application of large quantities of precultured marine hydrocarbonoclastic bacteria has not been very successful. A more effective measure for major spills seems to be through stimulation of the growth and activity of indigenous hydrocarbonoclastic bacteria with the application of lipophilic nitrogen-phosphorous–rich fertilizers, both in the open sea as well as on rocks and beaches contaminated with crude oil (*29*).

Oil spills in arid terrestrial environments are accompanied by the simultaneous occurrence of high pH, high salinity, and high loads of toxic organic compounds. In general, adaptation to osmotic stress under high salinity and pH requires increased intracellular salt concentration or accumulation of organic osmotic solutes (*30*). At elevated salinity, the microbial cell surface tends to become more hydrophilic, which will further limit physiological activity on hydrophobic hydrocarbons. High salt concentrations are also characterized by reduced dissolved oxygen, but some organisms can metabolize oil under these conditions, although the mechanisms are not well understood. Successful large-scale bioaugmentation has been implemented in a water pit (3600 m^3) heavily polluted with crude oil in northern Oman, where the addition of halophilic cultures reduced hydrocarbon concentrations from 10 to 40% (w/w) to below 1% (w/w) within a year (Fig. 3) (*31*).

Resistance to low pH and high concentrations of heavy metals

Metal extraction and metal leachate decontamination offers contrasting examples of microbial resistance and its potential use for bioremediation.

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Fig. 2. Bioaugmentation with OHRB. (**Left**) Injection of microbial cultures containing OHRB in an injection well or (**Right**) direct push injection without the use of wells in aquifers contaminated with chlorinated solvents. [Reprinted by permission from Springer Nature, (*22*).]

Bioextraction and recovery of valuable metals from sulfidic ores (biohydrometallurgy) depends on the activity of sulfur- and iron-oxidizing prokaryotes to solubilize the mineral pyrite (FeS_2) to H_2SO_4 and Fe^{3+}, during which protons and other metals trapped within the pyrite matrix are released. Biohydrometallurgic suspensions have extreme physicochemical characteristics, sometimes with negative pH values, and metal and sulfate concentrations between 10 and 100 g liter^{-1} (*32*).

Consortia of acidophilic prokaryotes used for biohydrometallurgy, mainly belonging to the genera *Acidithiobacillus* and *Leptospirillum*, are typically derived from natural acid rock drainage environments, such as the Tinto river in Spain, or from spontaneous enrichments derived from mine drainage. These acidophiles can grow at extremely low pH and high metal concentrations. Although growth at low pH has some advantages for cellular energy conservation because it builds a spontaneous pH gradient for the proton motive force across the cytoplasmic membrane, the protons still have to be neutralized within the cytoplasm. Some extreme acidophiles prevent ingress of protons by importing K^+ ions, which inverts the membrane potential (positive inside). They can also have highly impermeable membranes owing to the presence of tetraether lipids and specific membrane transporters, such as antiporters, symporters, H^+–adenosine triphosphatases (ATPases), or metal-transporting P-type ATPases, which remove excess protons and metal ions from the cytoplasm. Additionally, specific chaperones have been reported in acidophilic bacteria that stabilize DNA and proteins, which would otherwise be damaged by the low pH (*33*).

Metal leachates from mines are highly problematic because of their low pH, high sulfate, and high dissolved metal content. Sulfate-reducing bacteria (SRB) release sulfide, which will increase the pH and will react with the dissolved metal ions to precipitate in the form of poorly soluble metal sulfides. Stimulation of sulfidogenic activity has been tested in pilot-scale treatment of metal leachate from the zinc smelter Nyrstar in the Netherlands, and also for leachates from the gold mine Pueblo Viejo in the Dominican Republic. Both applications, however, required prior neutralization of the leachates before biological treatment. Nevertheless, acid- and metallotolerant SRB, such as *Desulfosporosinus acididurans* (*34*), have been isolated from low-pH environments and successfully deployed for initial biological leachate neutralization and subsequent metal detoxification in laboratory-scale reactors (*35*). The prior growth of acidophilic SRB in pH-controlled reactors may further improve the biological recovery of precipitated metallic sulphides and allow potential reuse in industrial processes (*36*).

Resistance to antibiotics and nonantibiotic biocides

Application of antibiotics and nonantibiotic biocides has increased dramatically in recent decades and has resulted in widespread selection of resistant or tolerant mutants. Resistance to antibiotics by the selection of RND efflux pump mechanisms can provide cross-resistance to a wide range of other adverse conditions and compounds. Hence, antibiotic resistance also frequently co-occurs with resistance to biocides and heavy metals. This results from the colocalization and/or comigration of genes conferring multiple resistance mechanisms (*37*, *38*). Antibiotic resistance genes occur in microbes in natural environments without obvious anthropogenic exposure to antibiotics. This indicates that they confer additional biological advantages (*39*), such as resistance to other environmental stressors or to interspecies competition strategies, and metabolism of toxic compounds structurally similar to antibiotics. Several previously unknown dioxygenases have been retrieved from soil metagenomic libraries screened for resistance against β-lactam antibiotics (*40*). These enzymes were also shown to transform other aromatic compounds (*40*). Some microbes can use these antibiotics as substrates for growth, although the mechanistic basis for this antibiotic subsistence has not been identified unequivocally (*41*).

Nonantibiotic biocides can also select for proliferation of resistant microorganisms capable of their biotransformation, as has been shown for a river sediment microbial community degrading benzalkonium chlorides (*42*). Strains of *Pseudomonas putida* and *Alcaligenes xylosoxidans*—which are capable of resisting high levels of the polychlorinated antimicrobial triclosan and using it as a sole carbon source—have been isolated from soil (*43*). Biocide resistance could potentially be put to good use—for instance, for biocides removal from the filters of drinking water treatment plants (DWTPs). However, success has been limited so far. Augmentation of *Aminobacter* sp. MSH1 to sand filters in recent pilot-scale studies of DWTPs only temporarily increased 2,6-dichlorobenzamide degradation. The loss of activity was attributed to starvation of the introduced bacteria because the micropollutant concentrations were low,

Fig. 3. Bioaugmentation with halophilic microorganisms. A bioaugmented open-air bioreactor in northern Oman (**Left**) just before and (**Right**) 1 year after seeding, as an example of hypersaline oil remediation technology. [Reprinted by permission from Springer Nature, (*31*).]

and metabolic competition occurred with more abundant assimilable organic carbon in the water (*44*, *45*).

Concluding remarks

The metabolic and stress-resistance traits that emerge in microorganisms in response to toxic compounds can be exploited for the bioremediation of spills of oil and chlorinated solvents, dissolution of valuable metals, and treating waste streams. However, designing sustainable bioremediation solutions, including those targeted at emerging micropollutants, is a major scientific challenge. The conceptual simplicity of bioaugmentation and attractiveness is deceptive, especially for single microbial strains (*44*–*46*). Microbiologists still have very little knowledge of the traits and conditions that need to be met to allow survival and population growth of nonnative microbes introduced into foreign ecosystems. The few studies that have measured the metabolic activities of inoculated bacterial strains in complex ecosystems have unveiled how divergent the biochemistry becomes in field conditions compared with the laboratory (*47*, *48*). Transposon library selection and sequencing have further shown just how many specific traits determine survival and proliferation in, for example, soil compared with the well-controlled conditions in the laboratory (*49*). Detailed experiments will be crucial for unraveling stress and resistance responses in inoculated strains and consortia and will be necessary to understand how productive metabolic traits can be deployed in order to functionally complement and restore contaminated ecosystems.

Genomic and allied technologies will permit better characterization of the prevailing resident microbial community in contaminated sites and inform community composition, xenometabolic potential, and adaptive capacity to adverse conditions. Meta-omic site diagnosis will provide inputs for advanced biogeochemical models (*50*, *51*). Such insights could be applied to diagnosing microbial communities for xenometabolic function at contaminated sites and for forecasting the success of specific measures, such as biostimulation or bioaugmentation, for accelerated bioremediation. Models could be expanded to address the potential roles of protozoan grazers and phage parasites that regulate microbial populations. For example, although phages can infect and eradicate populations of key detoxifier strains (*52*), they can also facilitate horizontal distribution of genes essential for bioremediation and as such promote degradation capacity.

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