AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews®

REVIEW

Evolutionary Pathways and Trajectories in Antibiotic Resistance

F. Baquero,[a] J. L. Martínez,[b] V. F. Lanza,[a,c] J. Rodríguez-Beltrán,[a] J. C. Galán,[a] A. San Millán,[b] R. Cantón,[a] T. M. Coque[a]

[a]Department of Microbiology, Ramón y Cajal University Hospital, Ramón y Cajal Institute for Health Research (IRYCIS), Network Center for Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain

[b]National Center for Biotechnology (CNB-CSIC), Madrid, Spain

[c]Central Bioinformatics Unit, Ramón y Cajal Institute for Health Research (IRYCIS), Madrid, Spain

(Continued)

Citation Baquero F, Martínez JL, F Lanza V, Rodríguez-Beltrán J, Galán JC, San Millán A, Cantón R, Coque TM. 2021. Evolutionary pathways and trajectories in antibiotic resistance. Clin Microbiol Rev 34:e00050-19. https://doi.org/10.1128/CMR.00050-19.

Address correspondence to F. Baquero, baquero@bitmailer.net.

Published 30 June 2021

SUMMARY Evolution is the hallmark of life. Descriptions of the evolution of microorganisms have provided a wealth of information, but knowledge regarding "what happened" has precluded a deeper understanding of "how" evolution has proceeded, as in the case of antimicrobial resistance. The difficulty in answering the "how" question lies in the multihierarchical dimensions of evolutionary processes, nested in complex networks, encompassing all units of selection, from genes to communities and ecosystems. At the simplest ontological level (as resistance genes), evolution proceeds by random (mutation and drift) and directional (natural selection) processes; however, sequential pathways of adaptive variation can occasionally be observed, and under fixed circumstances (particular fitness landscapes), evolution is predictable. At the highest level (such as that of plasmids, clones, species, microbiotas), the systems' degrees of freedom increase dramatically, related to the variable dispersal, fragmentation, relatedness, or coalescence of bacterial populations, depending on heterogeneous and changing niches and selective gradients in complex environments. Evolutionary trajectories of antibiotic resistance find their way in these changing landscapes subjected to random variations, becoming highly entropic and therefore unpredictable. However, experimental, phylogenetic, and ecogenetic analyses reveal preferential frequented paths (highways) where antibiotic resistance flows and propagates, allowing some understanding of evolutionary dynamics, modeling and designing interventions. Studies on antibiotic resistance have an applied aspect in improving individual health, One Health, and Global Health, as well as an academic value for understanding evolution. Most importantly, they have a heuristic significance as a model to reduce the negative influence of anthropogenic effects on the environment.

KEYWORDS antibiotic resistance, evolutionary biology, trajectories, pathways, evolutionary pathways, evolutionary trajectories

INTRODUCTION

The evolution of antimicrobial resistance (AMR) has been frequently reviewed in recent decades (1–4). We are trying to offer here a different scope centered not on the facts but on the processes determining these facts. The main objective of this review is to examine the causal (deterministic) and stochastic processes that have shaped the evolution of AMR. Pathways are sequences of genetic changes that form chains in which each step facilitates the next, favoring, step by step, a significant increase in AMR. However, antibiotic pathways explain only part of the trajectories of AMR, which flow for numerous reasons in addition to antibiotic selection, in many cases taking tortuous paths determined by chance, involving unlinked and arbitrary events, or determined by selective events unrelated to antibiotic exposure. The classic theory is that evolution progresses in accordance with general biological laws along evolutionary pathways, describing trajectories for different variants of organisms and genotypes, to reach, step by step, significant antibiotic-resistant phenotypes.

In fact, the truth is less clear and directional, an inescapable consequence of the complexity of the entities that influence AMR, which encompass various levels of biological hierarchies. Evolution cannot be traced along a single dimension (as a phylogenetic tree) but rather is the consequence of interactions in multiple dimensions, thereby resulting in multidimensional trajectories, following itineraries along a network rather than on a flat plane. This review is less concerned about describing "what happened" in the history of resistance (the descriptive "stamp collecting" of facts, the classic activity of biology, in the ironic statement by Ernest Rutherford) and more intent on the "how," covering the processes, mechanisms, and reasons shaping AMR.

Ernst Mayr made a distinction between proximate and ultimate causes in biology (5), using "proximate causation" to refer to the immediate factors (e.g., mutation and horizontal gene transfer) of processes and using "ultimate causation" with "final reasons" as the mechanisms causing the outcome (e.g., natural selection and evolution). The proximate causes constitute the chain of events that explain the final production of an effect, the "how," which in our case are the elements and processes creating the paths and trajectories that shape the current situation of AMR. The ultimate causes are the reasons explaining the evolution of these paths and trajectories.

From an anthropogenic perspective, AMR is a classical evolutionary process, based on a specific reaction (natural selection) by microbes to survive antibiotic exposure. However, AMR occurs in an extremely complex and variable ecobiological system encompassing the whole planet, involving numerous other causes (6). Causality should therefore be clearly differentiated from correlation alone (7). There are proximate and ultimate causes in AMR; however, the existence of causes does not imply logic in the evolution of resistance, which is a blind process based fundamentally on chance (8). This review therefore focuses on the proximate causes, paths, and trajectories and only occasionally discusses the primary drivers of such processes. By increasing our knowledge about paths and trajectories, we might eventually predict relatively close trends in AMR, paralleling meteorological predictions, which also consider chance and necessity.

ANTIBIOTIC RESISTANCE: BACTERIA AND GENES

Antimicrobial-Resistant Bacteria

From an anthropocentric, clinical standpoint, a bacterial organism is defined as antibiotic resistant when the chances of success when treating an infection produced by this organism with a specific antibiotic are low. For clinical purposes, susceptibility signifies treatability, which is based on the toxicological, pharmacodynamic, and pharmacokinetic properties of the antibiotic in question and on the clinical information from clinical trials and the cumulative experience of antibiotic success in treating particular infections. A worldwide effort to standardize criteria has led to the universal criteria for "resistance" (based on "breakpoints") for the various antibiotics (9). These breakpoints, which separate susceptible and resistant bacteria, are, however, mainly based on a single pharmacodynamic parameter: the antibiotic's MIC under standard defined *in vitro* conditions.

The criterion for an abnormal MIC level (compared with most strains of the species) can provide epidemiological cutoffs (ECOFFs) that define microorganisms with acquired resistance mechanisms as those that present MIC values above the upper limit of the normal distribution (wild-type population) in any given species or for any given compound, regardless of whether this information has clinical relevance (10–12).

The proposed operational definition for resistance (12) is based on the pairwise comparison of a parental strain with another derived strain either carrying an acquired putative resistance determinant or containing a mutation that alters its antibiotic susceptibility. If the parental strain is more susceptible than the derived strains, the acquired gene should be considered a resistance gene and the mutation a resistance mutation, irrespective of the resistance level achieved, which could help predict future trends in the emergence of resistance (13–15).

Based on the populational ECOFF definition of resistance, any microorganisms that falls beyond the normal MIC distribution for a given bacterial species should be considered resistant. From a clinical standpoint, we should distinguish between resistant bacteria (those that have acquired a resistance phenotype) and unsusceptible microorganisms (naturally, intrinsically resistant) before anti-infective therapy was available. Any bacterial species is naturally unsusceptible to some antimicrobials (e.g., Gram-negative bacteria are intrinsically resistant to glycopeptides) but can, under antibiotic selective pressure, acquire resistance to those antibiotics to which they were naturally susceptible.

Antimicrobial Resistance and Resilience Genes

The concept of the antibiotic "resistome" was proposed by G. Wright to describe the ensemble of genes present in a given habitat or bacteria and able to confer resistance to a certain antibiotic, that is, antimicrobial resistance genes (ARGs) (16, 17). Several studies have shown that any ecosystem contains its own ensemble of genes capable of conferring resistance in a heterologous bacterial host (18, 19). A few of these genes have previously been detected as having been acquired through horizontal gene transfer (HGT) by human pathogens, and the overall structure of the resistomes is linked to their phylogeny (20), indicating that most resistance genes present in microbiomes belong to the intrinsic resistome. These findings agree with studies on the intrinsic resistome of bacterial pathogens, which show that up to 3% of the bacterial genome might contribute to AMR (21). Considering the number of different species present in any given habitat and the diversity of microbiomes in various environments, there are likely millions of genes in nature capable of conferring resistance to antibiotics in a heterologous host.

In contrast, there are only a few hundred genes that have actually been acquired by human pathogens and constitute a risk for human health. There are then bottlenecks that restrict the entrance of ARGs into the population of bacterial pathogens, frequently linked to their hierarchical structure (22). The study of the AMR mobilome, understood as the set of resistance genes present in mobile elements (18), could help in the early detection of novel and potentially relevant resistance genes before they disseminate among bacterial pathogens. A key issue is the observability of AMR; i.e., something might exist but emerges only if the emerging entity achieves the abundance to reach the boundaries of visibility, which, in principle, implies growth as a prerequisite (23). In this age of advanced technologies, growth might become an increasingly less necessary condition, given the power of our analytical instruments potentially enabling the recognition of the first bursts of emergent phenomena in AMR.

The concept of ARG can be afforded under two different frameworks, from ecological and evolutionary to clinical viewpoints, First, ARGs have evolved to counteract the activity of antibiotic producers (24). Antibiotic producers must also have detoxification systems that serve to counteract the activity of the antimicrobials they produce. Although detoxification systems should not be considered bona fide resistance genes given that they do not serve to resist a competitor, it has been suggested that they can be progenitors of ARGs acquired from human pathogens no matter the original function they were selected for. From an anthropogenic, clinical point of view, an ARG is the one that increases resistance when is present or is expressed at higher levels or increases susceptibility when it is inactivated, ARGs were present in the microbiosphere

before the anthropogenic release of antimicrobials (25, 26), which explains the presence of ARGs in pristine soil (27). Most likely several ARGs were not born as resistance genes but as genes that encode basic functions of cell machinery. There are, for example, responsible for the seemingly infinite variety and ubiquity in the bacterial world of modifying enzymes, such as acetyltransferases, methylases, nucleotidyltransferases, esterases, phosphorylases, peptidases, thioltransferases, hydroxylases, glycosyltransferases, and oxidases. Modifying enzymes act in a diffuse manner on multiple targets, contributing to phenotypic versatility (28). These functions have the potential to reduce inhibitory activity or inactivating natural antimicrobials, and antibiotic exposure has likely contributed to the evolution of these genes by forming efficient ARGs. Further, significant resistance genes can be conceived of as "exaptations" in which a sequence coding for a particular function may acquire another function just because the context, not the gene, changes (29). The function of resistance is acquired just as the gene becomes decontextualized in a new host (30).

In a universe of potential resistance mechanisms, everything depends on selective events. Expanding on the classic Baas Becking hypothesis, "every gene is everywhere, but the environment selects" (31), the antibiotic might have a chance encounter with one of these preexisting gene-encoded functions; perhaps this coincidentally provides a certain inactivation of the antibiotic compound. In this case, the bacterial organism expressing such a function (certainly with a purpose other than resistance) will increase in relative fitness in the presence of the antibiotic (i.e., it will be selected). If the exposure is frequent, the selected function should increasingly augment the genes' ability to detoxify the antibiotic, closing in on an efficient ARG. This process of emergence of resistance genes can be accelerated by combinatorial events involving the building up of complex (chimeric) proteins from sequences determining protein domains, i.e., protein sequences able to evolve and function independently (32) and also combinations of preexisting genes (see below). The possibility that antibiotic resistance genes might also emerge as *de novo* genes, i.e., new genes derived from changes of the noncoding segments of the genome (32, 33), is almost unexplored (34). Random sequences can also evolve rapidly into *de novo* functional promoters (35), eventually increasing ARG expression.

Resilience is the property of a system to return to a stable state following a perturbation (36). During antibiotic exposure, the biodiversity of the microbiota is altered. Intrinsic resistance might provide the microbiota the adaptive capacity to recovering from deep antibiotic perturbations; ARGs may have a role in such recovery. AmpC beta-lactamases from enteric gammaproteobacteria, or many efflux pumps from these and other pathogens, which provide resistance to beta-lactam agents, have evolved in mammalian gastrointestinal systems over millions of years (37, 38). Distinguishing resilience genes within the overall resistance genes might aid the analysis of the risks associated with the presence of these genes in a microbiota, which are currently grouped together and ranked similarly. Resilience genes are "markers" of the normal microbiota, and variation in the content of resilience genes might influence the stability of bacterial communities (39). If massive exposure to anthropogenic antibiotics has altered the effectiveness of resilience genes in improving the detoxification activity of commensal organisms, then the blurring of the distinction of resilient genes within resistance genes could be a key field of research that has been scarcely explored. However, this blurring occurs, for instance, in the case of mutations widening the substrate spectrum of AmpC beta-lactamases (40, 41).

EVOLUTION: UNITS, TOPOLOGIES, AND TRAJECTORIES

The term evolution originates from the Latin word *evolutionem* (to unroll a scroll book), thus providing a highly suggestive image of gain of information and adaptation. Essentially, what we perceive now can be explained by what came before. As previously noted, our interest is less what happened in the evolution of AMR than how and, more obscurely, why it occurred. To study the how and why implies the possibility that the evolution of AMR can in fact be understood—in other words, whether the evolution of AMR can be predictable. The major difficulties in predicting AMR are related to (i) the complexity of the biological and environmental components shaping AMR and

(ii) the influence of the randomness of biological and environmental processes on the evolutionary uncertainty of resistance (42). Evolution is a stress-reducing process, and the engine driving it consists of the potential difference between an organism's current fitness and the possibility for better fitness, to thereby bring it more in balance with its environment. This difference can be expressed as a difference in stress, with equilibrium generally being awarded with lower stress and successful replication.

The Units of Evolution: Evolutionary Individuals

The nature of units of evolution (the evolutionary individual) is critical for understanding AMR processes and trajectories (43, 44) (Fig. 1 and 2). Trajectories of which kind of biological objects? There should be a network of paths associated with the evolution of different types of individuals, biogenic units (45) with growing information complexity, from molecules to organisms and communities. How can we approach the identification of evolutionary individuals, the biological units sequentially modified in time by natural selection? As a condensation of the concepts of Stephen Jay Gould (46, 47), there are four minimal criteria to define an evolutionary individual: reproduction, inheritance, variation, and interactive relations, which occur from the lowest hierarchies, starting with genes. However, evolutionary individuals also encompass larger sequences (such as operons), cellular genomes, mobile genetic elements (MGEs) (such as phages, transposable units and plasmids), cells, clonal populations, species, multispecies assemblies, and holobionts (hosts and microbiota as single biological entities) (48–50) (Fig. 1 and 2).

The key concept is that these evolutionary units are individuals that can evolve independently but are frequently embedded in each another, resulting in the integration of lower-level replicators into high-level replicators. At each step, this integration constitutes a novel individual, with particular adaptive needs and possibilities for coniche construction (51, 52) which occurs asymmetrically, following hierarchy-selected events. Therefore, the evolution of any unit at any level of the hierarchy might influence the evolution of all others, both in a top-down and in a bottom-up dynamic, creating a complex multidimensional landscape where the evolution of AMR flows along hierarchies.

Topology of Antimicrobial Resistance Trajectories

Evolutionary paths of AMR (a collection of phenotypes) occur within a complex space of G-types (genotypes, genomotypes, and metagenomic types) corresponding to the whole variety of evolutionary individuals, the different units of selection (Fig. 2). The accessibility of a phenotype is represented by genotype-phenotype maps (53), which determine how phenotypes vary with genotypes. As represented in Fig. 3, any evolutionary individual has a (clonal) descent that is represented by a cylinder, a tube that progresses in time. There are internal changes in the clonal lineage (such as mutations) that provide changes, so that the trajectory of changes occurs inside the tube (the space of variation), in synchrony and sympatry with many other lineages. Different neighbor cylinders might exchange characters by horizontal transfer (e.g., genes), which are now introduced into other cylinders and influence the vertical descent inside these tubes. A set of tubes exchanging adaptive characters should tend toward ecological convergence, increasing interactive relations.

A realistic description of evolutionary trajectories of AMR should include a complex transhierarchical network of trajectories encompassing entities at various levels, from proteins to populations and communities (54, 55). The evolution of AMR should be necessarily compatible at any level of the hierarchy with other evolutions, other trajectories in search of numerous other types of adaptive advantages unrelated to AMR. These adaptive needs can eventually conflict with the evolution of AMR, and their paths might eventually converge during part of the journey (acquisition of traits that are advantageous for the adaptive needs of both organisms). For instance, traits favoring *Escherichia coli* gut colonization, as the production of microcins (small antimicrobial peptides) is frequent in multiresistant clones, such as O25B-ST131. In general, any adaptive gain, modification, or loss of metabolic pathways should influence antibiotic susceptibility (as a bactericidal effect) and resistance (56, 57).

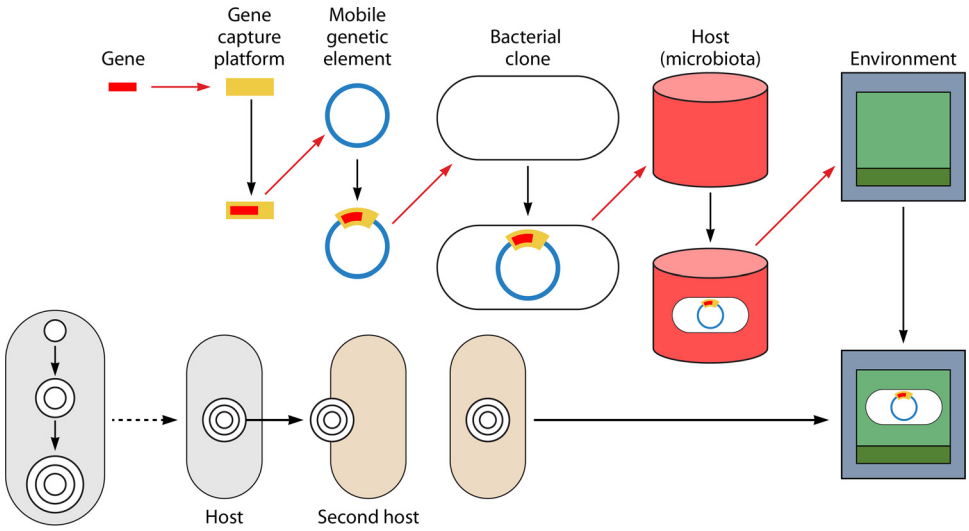


FIG 1 The basic nested structure of the evolutionary units involved in antibiotic resistance. From left to right, a resistance gene is caught by a gene capture platform (as an integron), which might, in turn, be inserted into a conjugative mobile genetic element (as a plasmid), which is acquired by a particular bacterial clone. This clone is inserted in the host microbiome; the host is part of an environment where the resistance gene contributes to the environmental resistome. Evolutionary units are units of selection, i.e., they can be independently selected. The small diagram at the bottom left shows that all of these successive steps are due to internal (cellular) *cis*-acting transmission events (resulting in concentric rings), followed by unenclosed *trans*-acting transmission events (clone with resistance plasmid, host microbiota, or the environment), for example, when a bacterial cell containing a plasmid and a gene (concentric rings) is transmitted from a human host to another host and then to the environment (black line).

However, the evolutionary trajectories dominated by resistance (to antibiotics, biocides, or metals) might have certain advantages over other trajectories, given that the selective effect is stronger. Observations in other fields have suggested that in the case of conflict, the evolutionary side that can survive and grow at the expense of others (antagonism) is able to adjust the variable in its preferred direction (58). Interactions (such as competition) between trajectories might occur between successive alleles on the same trajectory (636); variants with a high initial fitness might have less fitness later and might be outcompeted by other variants (59).

Are there random trajectories? AMR evolves through processes that involve determinism, stochasticity, and random drift. Drift evolution implies that a number of variant phenotypes (in this case, resistance phenotypes) in a population have emerged and spread by reasons completely unrelated to the microorganism's adaptive needs when exposed to antimicrobial agents or to other adaptive needs. These variants can be hooked by antibiotic selection and enriched by drift in small populations. In the first edition of *On the Origin of Species*, Charles Darwin indicated the possibility of fluctuations in the frequency of variations with no adaptive significance, at least at the moment of their emergence (60). Paradoxically, such observation was the foundation of the concept of non-Darwinian evolution (60). Sewall Wright was the first to attach this significance to random drift and small, newly isolated populations through his shifting balance theory of speciation. Drift, stochastic introgression, and hybridization events produce "hopeful monsters," overcoming the need for gradual changes in evolutionary trajectories (61, 62), eventually giving rise to high-risk resistant bacterial clones.

Dispersal provides adaptive chances for minorities. Random drift is frequently presented as a sampling effect, such that the sampling of a population at different locations might yield differing results in the frequency of particular variants. Large bacterial populations mostly evolve deterministically, whereas small populations follow more stochastic evolutionary paths (62). Drift is a powerful process in the formation of species (62, 63), which is also true for the clonalization processes inside bacterial species. Bacterial dispersal distributes small populations over space, eventually leading to spatially structured populations

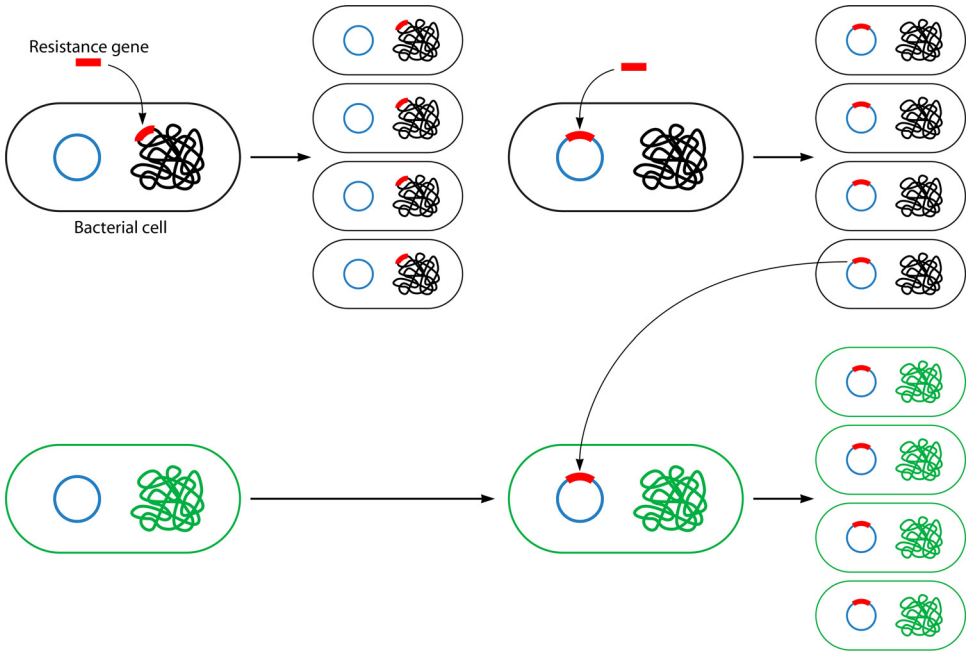


FIG 2 Units of selection as evolutionary units. A bacterial cell and a conjugative plasmid carrying antibiotic resistance genes constitute different evolutionary units, given that they are independent beneficiaries. At the top, a resistance gene that is externally acquired (small red rectangle) by the cell can be integrated either in the chromosome (black string ball) or in a conjugative plasmid (blue ring). In a selective event, the cell with the red gene in the chromosome reaches 4 copies, but the plasmid is independently transferred to a different bacterial cell (green), which is also selected and reaches 4 copies. At the end, the balance for each type of cell is 4 copies, with 8 copies for the plasmid, indicating that under this single selective antibiotic event, the plasmid is a better beneficiary than any of the bacterial cells hosting it; in other words, the plasmid is an independent unit of selection, a different evolutionary unit.

colonizing different environmental patches. A genetic variation allowing access to an antibiotic resistance phenotype might have a significant biological cost when competing with the wild progenitor population and will therefore be prone to extinction in the absence of antibiotic selection, but not in isolation.

When is drift evolution of AMR expected to occur in practice? The main conditions are a reduction in population size by spatial-temporal fragmentation and opportunities for growth of the reduced groups in favorable patches, forming metapopulations. Fragmentation of infective populations occurs due to a number of factors. First, in host-to-host transmission processes, a small propagulum of cells serves to initiate colonization or infection in each new host; thus, a spatially structured fragmentation of the original population should occur. Second, further fragmentations occur within the individual host, where bacterial invasion is necessarily linked with the dilution of the offending organisms in different compartments, tissues, and cells, and inflammatory processes frequently lead to sequestration of bacteria in particular locations. Drift-generated resistant variants might therefore eventually multiply locally. An increase in the number of colonizable subhabitats is expected, especially in chronic infections, and when foreign bodies are present, the increase in the number of colonizable subhabitats is an expected issue. Biofilms, which are frequent in chronic infective processes, provide spatial structuration of bacteria, facilitating the drift evolution of resistance (64) and diversification at large (65). Third, the release of human and animal sewage and other wastewater into the environment produces dilution and population fragmentation. The attachment of bacterial cells to soil particles (66) and organic remains increases the frequency of independent minipatches. Fourth, the use of antimicrobial agents and their release into the environment can reduce the size of bacterial populations and promote the evolution of drift-promoted resistance to different antibiotics, even in the absence of selection; this is certainly a scarcely treated topic. Fifth, fragmentation can occur due to asymmetric (specific) mechanical forces, affecting bacterial cell adhesion to particular surfaces (67).

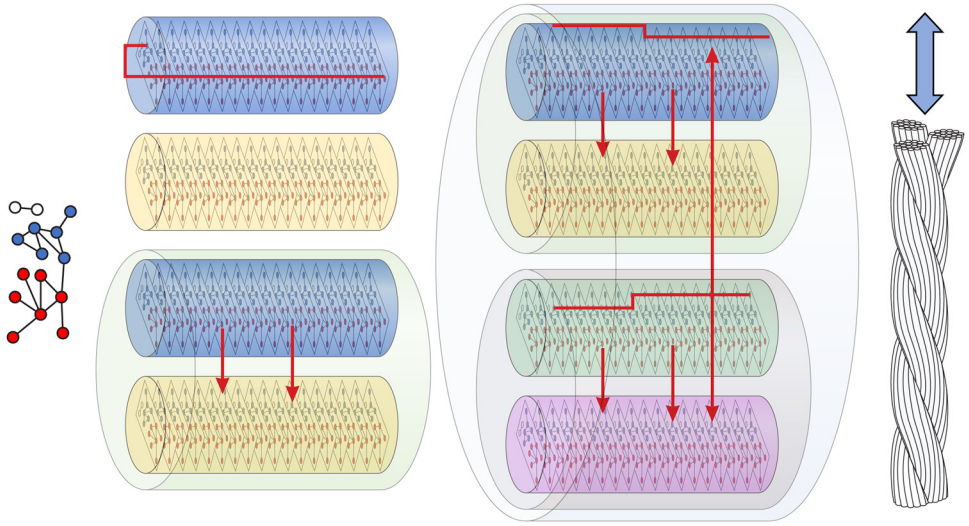


FIG 3 The topological interactions of bacterial populations in space and time: from clones to spinning evolutionary trajectories. Bacterial species have a complex population structure consisting of clonal ensembles linked by phylogenetic relations, which can be represented as a network in a plane (left). These clonal ensembles are sequentially maintained (left to right), but there is the possibility of clonal variation or recombination over time (red arrows). The structure of each bacterial species is frequently in the neighborhood of other species with their own structure. This vicinity is represented by a larger cylinder consisting of both of the species (middle) and enables horizontal genetic interactions (red arrows). In complex ecosystems (such as microbiotas), several cylinders are ecologically and functionally integrated, facilitating genetic exchange among apparently distant lineages (lower section). The interactive spinning of different evolutionary strands results in a single evolutionary material, which can be represented as a rope, based on vertical and horizontal interactions (red lines), giving rise to twisted common trajectories; however, the components can eventually be untwisted in changing environments (blue bidirectional arrow). The concept depicted here is that the events resulting in antibiotic resistance influence not only the trajectory of a particular clone or species in which they emerge but also the trajectories of complex bacterial ensembles.

Drift, draft, and trajectories. As stated before, the contribution of drift to AMR is akin to that of mutational events, offering random genetic variants to the hook of selection. Drift might therefore complement directional evolution mediated by successive adaptive benefits, providing random solutions in broken adaptive trajectories (68). Fitness plains and valleys (and not the peaks) are the territory of drift (69), where low density, neutrality. and near neutrality dominate, providing the substrate for adaptive and hidden, preadaptive evolution (Fig. 4).

However, even if a large population is not dispersed in small populations, neutral variation can be maintained because it is randomly linked to selectable traits and frequently "hitchhikes" with those loci subjected to directional selection. That means that the adaptive variation in a selectable locus can therefore induce stochastic dynamics (resembling genetic drift) at a closely linked neutral locus; such hitchhiking is termed genetic draft (62, 70). The randomness of early stages in many evolutionary trajectories leading to AMR is a consequence of drift, but once the adaptive trajectory starts, with low increases in fitness, directionality (selection) eventually tends to be imposed.

Genealogical, across-network, and spinning trajectories. Adaptive trajectories follow the fundamental tenet of evolution, the Darwinian principle of "descent with modification" (71, 72). The study of phylogenies is therefore essential in classifying evolving individuals by similarities and tracing the process (trajectory) of their relationship with common ancestors. A limitation in the current phylogenetic (genealogical) analysis is the bias imposed in databases by the predominance of organisms of particular interest (such as those with AMR) and the almost total absence of "real last-common evolutionary ancestors" (73). The phylogenetic approach might indicate the presence of an evolutionary trajectory within a single progeny (genealogy) but needs confirmation with actual data, a task that could be facilitated by automated phylogenetic tools, eventually associated with recombinational analysis (73, 74). The basic problem with this approach is that lineage-only-based phylogenies of bacterial organisms

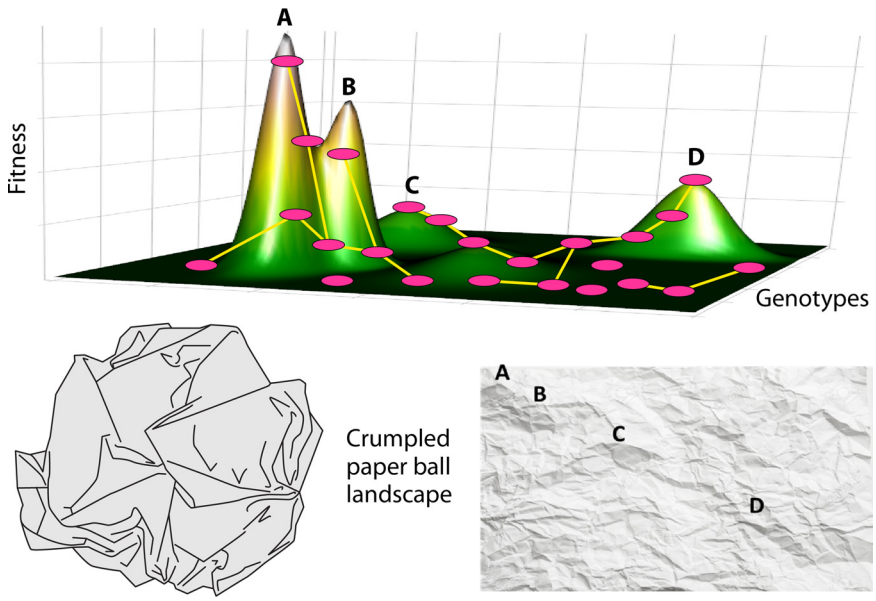


FIG 4 Fitness landscapes in antibiotic resistance. At the top is an image of the classic fitness landscape metaphor developed in 1932 by Sewall Wright, where in a bidimensional plane (black) different genotypes are represented, their corresponding height in the vertical axis showing the fitness of each genotype (reproductive success) under the conditions of the landscape. Red ovals correspond to the variation (for instance, mutation) from one genotype to another one (yellow lines). Note that series of mutations (pathways) might reach low (C), medium (D), or high (A and B) fitness peaks (for instance, reaching very high MICs), but some of these pathways might have originated just by random drift (without natural selection) in the flat area of the landscape. If this landscape is crumpled as a paper ball (bottom left), peaks can go into proximity, and the genotype selected into a peak can have access to other fitness peaks (eventually resulting in genetic recombination or exchange). At the bottom right, the paper ball is deployed to illustrate the fitness landscape.

are likely corrupted in nature by the high frequency of introgressive events, leading to the stable integration of genetic material from one bacterial species into another. HGT is essential for "building the web of life" by associating different genealogical lineages (75), which is true for every evolutionary individual along the hierarchy, from genes to communities. The representation of these intergenealogical branches produces not a more tree-like pattern but rather a reticulate network pattern, which likely reflects the space of evolutionary trajectories in a more integrative manner (76) (Fig. 5 and 6). Network-based approaches, such as sequence similarity networks, gene networks, genome networks (including core genome, accessory genome, and regulatory genome networks), family networks, genus networks, and genome bipartite graphs, are frequently employed in current evolutionary studies (77).

Are "tree-phylogenetic" and "network-reticular" trajectories mutually exclusive? In many cases, it is still possible to make robust phylogenetic inferences even in light of substantial HGT (78, 79). Horizontal linkages can be hypothesized between vertical phylogenies, creating a superphylogeny. The linkages can be thought of as resembling distinct wool fibers, combed together with other strands which are in contact with each other, create a single rolag (roll) of wool. Spinning produces the interwoven fusion of strands into a single evolutionary material composed of vertical and horizontal interactions, giving rise to a cord or spinning trajectories (Fig. 3).

Diversifying and unifying evolution. Evolution progresses over time; in fact, the goal of evolution is the conquest of time. Evolution, if not replication alone (80), leads to progressive diversification (diversifying evolution); i.e., producing numerous variants from a single structure many variants are produced, an "*ex unibus plurum*" disruptive dynamics. Resistance genes, transposons, plasmids, resistant clones, species, and communities are subjected to constant diversification, while these variants (or at least the variants that have survived) simultaneously tend to aggregate to form complex configurations with greater evolutionary possibilities (resulting in a unifying evolution); i.e., a single superstructure

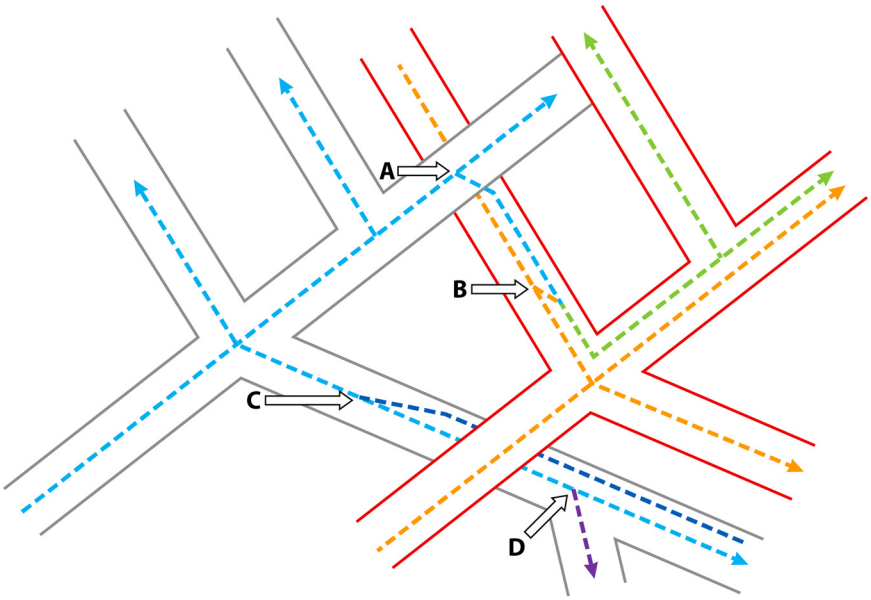


FIG 5 Phylogenetic networks and antibiotic resistance: trees within trees. Two separated phylogenetic networks (gray and red, either from clones, species, families) are superimposed. Inside the branches, the mobile genetic elements (MGEs) carrying antibiotic resistance genes coevolve with their hosts. Arrows represent the various events that modify the evolution of MGEs. (A) The light blue MGE introgresses (i.e., conjugates) from the gray to the red tree. (B) The recombination with the indigenous MGE (yellow) creates a new MGE variant (green), which eventually evolves within a separate branch of the red tree. (C) A variant (dark blue) of the indigenous (light blue) MGE of the gray tree emerges (mutation, internal recombination). This variant can segregate into a new branch of the gray tree (D). The purpose is to show the mixture of MGE-bacterial associations and the eventual modifications of their coevolution, giving rise to novel MGEs able to colonize other bacterial branches.

emerges from numerous diverse structures, an "*ex pluribus unum*" integrative dynamics (81, 82). This is a biphasic universal game of rapid expansion-inflation and slow (but creative) contractions, resembling other evolutionary patterns in physics; in fact, this system can be presented as an entropic-antientropic dynamics.

Unifying or integrative evolution should improve robustness, i.e., a configuration's ability to tolerate changes that might become deleterious for survival. Unifying evolution ensures stability, long-term exploitation of resources despite alterations, and niche construction and might increase the selection of integrated "wholes." Unification is a synthetic dimension, where evolution improves the quality and efficiency of the evolutionary constructions, obtaining all possible advantages from the exploited area; in this sense, it is a K-strategy, favoring reproduction in the temporal dimension. Both dimensions are pivotal in the evolutionary "density game" theory (83).

The r/K selection theory indicates that bacterial populations might reach a certain equilibrium (trade-off) between disruptive and unifying evolution, ensuring a balance of reproduction (quantity) and carrying capacity with complex local specialization (quality). This equilibrium has been predicted to occur in antibiotic-resistant populations (84). These disruptive-integrative evolutionary dynamics imply the possibility of breaking robustness (leading to a novel round of diversification). In complex network systems, there is the possibility of asymmetrical dynamics in one part of the complex, giving rise to system clashes (85).

SOURCES OF VARIATION

Phenotypic Variation: Bet-Hedging Adaptive Strategies

In bacterial populations, the continuous emergence of minorities of phenotypic variants produces significant phenotypic heterogeneity (plasticity), which, due to the subdivision of risk spreading, helps increase the lineage's chances of survival when confronted with unpredictable environmental fluctuations (86). In most cases, the origin of the heterogeneity appears to be derived from "noisy gene expression," random epigenetic interactions, gene

amplification and, with less stochasticity, reversible stochastic switching of gene expression (bistability) (87, 88). There should be a certain "cost of high phenotypic variation" dampening the strength of selection toward phenotypic heterogeneity and promoting directional selection of certain trajectories (89). However, a high rate of phenotypic heterogeneity is a safe "emergence strategy" for bacterial survival, but the advantageous phenotypic variants do not necessarily guide the directionality of the genetic adaptive trajectory (90). Such a strategy has been presented as "bet hedging," where certain phenotypes are selected under differing conditions and at different times, even though in most other cases the phenotypes can reduce the variant's fitness (91). The bet-hedging strategy occurs in antibiotic heteroresistance, when a minority "resistant" subpopulation is present within a main population of susceptible cells (92).

Functional or genetic variations of the global regulators (as ArcA or RpoS) probably play an important role in global one-step adaptation (93). Micronutrition and other environmental effects might affect the evolution of AMR. In certain cases, "bet-hedging" can also act as a nonstrategy, governed only by fortuitous (not inheritable) errors in cell replication, resulting in transient periods of nonreplication and/or slowed metabolism, as likely occur in certain "persister phenotypes" (94). Such "cellular noise" is likely amplified by epigenetic inheritance, stochastic transmission of proteins, RNAs, and other biomolecules from parent to offspring cells (95).

We previously discussed errors in translation as a source of "phenotypic mutations." Amino acid misincorporation during translation produces mutated proteins that might produce novel functions, including AMR. Erroneous protein synthesis might affect the protein's specific activity, such as misfolding and stability, with possible phenotypic consequences. The frequency of noncognate amino acid incorporation is as high, in the range of 10^{-4} to 10^{-5}. One-fifth of proteins produced in a given cell contain at least one wrong amino acid (96); however, considering the proteins' short lifetime, these changes can have phenotypic consequences over a short period (97), which can be sufficient for expressing an antibiotic-tolerant phenotype, particularly under conditions of slow growth (98). In summary, phenotypic noise is a potentially important factor in evolution (8). Phenotypic plasticity and fluctuation accelerate evolutionary rates in multipeaked landscapes (Baldwin effect) (99).

Mutation Rate and Antimicrobial Resistance

Mutation essentially depends on the error rate of replication set by the accuracy of DNA polymerases and various DNA repair systems. In most DNA-based microbes, the mutation rate ranges from 10^{-10} to 10^{-9}/cell/generation, depending on the specific substitution, gene, and organism and considering selectively favorable, unfavorable, or neutral mutations. This rate is approximately 10 times lower than the typical frequency of mutation (e.g., 10^{-8} for *E. coli*), which measures all mutants present in a given population as those surviving a given antibiotic concentration (100).

Simple calculations offer an intuitive image of the mutation frequency in natural populations. The *E. coli* genome has approximately 5,000 genes, and the mutation rate for wild-type *E. coli* is 1×10^{-3} per genome (cell) per generation, which, divided by the number of genes (0.001/5,000), yields 2×10^{-7} per gene and (cell) generation. If there are 10^9 cells/ml in the colon in a volume of 1,000 ml, there would be 10^{12} *E. coli* cells in a single host, implying that there are 200,000 mutations per gene per day (1 generation) for *E. coli* in a given host. Given that particular *E. coli* clones are frequently stable colonizers of the gut, thousands of generations will amplify the total number of possible mutations. Similar calculations regarding the rate of evolutionary change have been recently discussed in relation to the gut microbiome and other *in vivo* conditions (101, 102).

The rate of mutation per gene is not linear across the chromosome; there are genes and regional mutational hot spots such as slippage contingency sequences (103). These privileged variations have been shown to compensate for the limitations of host-to-host transmission bottlenecks (104). Mutation densities are greatest in regions predicted to have high superhelicity (105). In any case, our current ability to detect rare mutations in the global sequence space that potentially provide AMR is probably low.

Increasing mutation rates can be expected to offer a wealth of novel mutations that eventually can produce selectable AMR phenotypes. Approximately 1% of *E. coli* strains have at least 100 times the modal mutation frequency of 10^{-8} (strong mutators). A very high proportion of strains (11 to 38% in various series) had frequencies exceeding 4 times, in some cases 40 times, this modal value (weak mutators) (106). A mutator allele and its potential beneficial mutations arising from hypermutability are physically and genetically associated in the same chromosome. As a result, the mutator allele will hitchhike with increased frequency in the population together with the beneficial mutation. In populations of sufficient size, advantageous mutations tend to appear in normo-mutators, and the selective process will therefore enrich low-mutating organisms. Eventually, the adaptive success of normo-mutators might prevent further fixation of strong mutators. However, not all mutators are equally likely to produce a given mutation. This bias emerges from the molecular mode of action of the mutation correction system that is disrupted in each mutator genotype. For instance, inactivation of the mismatch repair system in *E. coli* leads to a specific ~100-fold increase in G:C → A:T and A:T → G:C mutations (107), which has profound implications for the competitive ability of mutators and determines their evolutionary success (108). Experimental evolution research has demonstrated the possibility that the emergence of a mutator might occur under antibiotic exposure by the reversible insertion of a mobile element to inactivate *mutS*, resulting in several mutations independently able to increase resistance (at various levels) to a challenging antibiotic in the population, thus providing an "efficient survey" of potentially successful evolutionary pathways (109). It has been shown, both in the case of mutation-based resistance (110) and in the evolution of resistant genes carried by MGEs (111), that antibiotic-resistant organisms frequently have increased mutation rates, which suggests the evolutionary consequences of hypermutation. Does the fact that organisms with mutator alleles can hitchhike with antibiotic resistance phenotypes indicate that the rise in AMR might increase the evolvability of bacterial populations in general?

The methods by which bacteria modify mutator phenotypes are certainly of interest (112). It has been shown that a number of hypermutable organisms evolve to phenotypes of normal mutation rates, eventually by reacquisition of the functionality of damaged mismatch repair systems or by the coincidental overexpression of mechanisms that reduce the endogenous mechanisms of mutation (74). Due to these effects, populations with lower fitness but more robustness to mutational effects might displace highly replicative hypermutable populations, which has been described as "the survival of the flattest" (113, 114).

Mobilization of insertion sequences (ISs) in particular and transposable elements (TEs) in general causes genomic variability in bacteria (115, 116); however, their influence on mutation rates and adaptive evolution is small compared with that of mismatch repair mutator alleles. There is competition between mismatch hypermutation and IS propagation by hitchhiking (117). Genomes have therefore evolved suppressors that limit transposon spread (118).

Polyploidy and Gene Amplification

As discussed in the previous section, increased copies of a particular gene (polyploidy) should increase the possibility of mutational modification and evolvability in general. Bacterial stress (including antibiotic stress) might produce cell filamentation and polyploidy. Gene amplification (gene duplication in its simplest version) is likely relevant in the adaptation to antibiotic exposure because it generates extensive and reversible genetic variation on which adaptive evolution can act. The steady-state frequencies of gene duplication are extremely high, typically ranging between 10^{-5} and 10^{-2} per cell per gene, and the importance of gene-dosing effect has been shown in sulfonamide, trimethoprim, glycopeptide, and beta-lactam resistance (including resistance to beta-lactam–beta-lactamase inhibitor combinations) (119, 120).

The genes that are present in high-copy-number plasmids are also "amplified" and therefore increase the probability of new adaptive mutations, eventually leading to

higher levels of resistance (121). Fitness costs have been evaluated, and each additional kilobase pair of DNA reduces fitness by approximately 0.15%, resulting in amplification returning to the original single-gene status. No signal of this transient event will remain in the genome sequence, which is why this evolutionary mechanism remains underdetected (122). However, the high prevalence of antibiotic heteroresistance in pathogenic bacteria is most likely caused by gene amplification (123).

An interesting topic is the role of MGEs in evolution through polyploidy or gene amplification. Self-replicating MGEs control their own copy number in the host cell. Some of these elements, such as the ubiquitous small multicopy plasmids, usually present 10 to 20 copies per cell. Plasmids therefore represent a potential platform for the neofunctionalization of genes that could easily overcome Ohno's dilemma (gene duplication is selected because of an increase in the original function, but then the new copy is not free to be evolve to have another other novel function) (124). A high number of plasmid-borne gene copies would allow bacteria to explore new functions through mutation while conserving the functional backup of several copies of the gene (125).

Horizontal Gene Transfer

HGT provides the theoretical possibility for each gene of the biosphere to enter in contact with the genome of any bacterial organism. A conservative evaluation would indicate that there are 10^8 different genes in the world capable of conferring resistance to antibiotics, a number obviously beyond our analytical capability. This potential commonality is based on the fact that even remote possibilities might occur, sustained by the astronomically large number of bacteria (3^{29} cells) estimated to exist in the world. In a strict sense, ARGs (not including wild or mutated genes providing physiological functions) belong preferentially to the acquired (accessory) class of genes (12). Transferred genes are concentrated in only approximately 1% of the chromosomal regions (hot spots) (126), which is likely one of the key roles of extrachromosomal elements in integrating adaptive genes. Even if accepted, the genes might be unable to function as significant pieces of information, such as providing an AMR phenotype. Disparity in codon usage between the donor and recipient organisms can influence gene translation efficiency and might impose a fitness cost for the receptor.

There is a large spectrum of related transposable elements that are vehicles for ARGs. In addition to resistance genes, transposons might carry other adaptive elements that can help in the selection of AMR. Notably, the Tn*3* family of transposons can capture (or evolve) entire operons, with resistance to heavy metals (such as mercury), AMR, breakdown of halogenated aromatics, or virulence. The effects of transposable units such as IS include massive expansion and loss of DNA fragments, producing gene inactivation and decay, genome rearrangements, and genome reduction (116). How is this turmoil tolerated? There should be a way of regulating the genome's optimal size. Frequent HGT leading to genetic innovation is probably compensated for by highly frequent gene loss, leading to genomic contraction (127). It can be argued that the acquisition of high pathogenicity and AMR islands could be favored in variant clonal backgrounds having experienced genome reduction. Occasionally, large chromosomal deletions might produce a growth advantage in the presence of an antibiotic, as in the case of *Pseudomonas aeruginosa* and meropenem or ceftazidime resistance (128, 129).

Does the anthropogenic release of antibiotics and the resulting spread of transferable AMR act as a driver (accelerator) of microbial evolution? Under antibiotic exposure, genetic promiscuity is expected to increase. Recombination events are also expected to contribute to the long-term adaptations of resistant populations in changing environments (complex fitness landscapes) interacting with stochastic epigenetic variation (130). Cells have a wide variety of protective mechanisms to limit dangerous recombination events originated by the acquisition of foreign DNA, even if such DNA might be helpful, as in the case of AMR. Restriction modification (RM) systems and CRISPR (131, 132), frequently located in "defense islands" in microbial genomes, are the main post-transfer sequence-directed immunity mechanisms protecting a given host cell from invasion by foreign DNA.

VARIATION SHAPING TRAJECTORIES UNDER ANTIBIOTIC EXPOSURE

Stress and Antibiotics as Drivers of Genetic Variation

Stress-induced mutagenesis is a main driver of bacterial evolution (133). Antibiotics are not only selectors but also drivers of bacterial genetic variation. Antimicrobials produce stress reactions in susceptible organisms, frequently at subinhibitory concentrations, during growth phases in which antibiotics are less active or during at least relatively short periods. Bacterial stress is likely the result of conflicting cellular signals: on one hand, positive signals "to grow," and on the other, signals indicating the "impossibility to grow." Frequently, that results in transcription-translation conflicts, with formation of R-loops (because of backtracking of RNA), resulting in DNA damage and eventually being lethal (134). The mutation rate can be increased by antimicrobials promoting the stress-induced SOS response, which modulates genetic instability (135). First, stress frequently results in bacterial filamentation and cellular polyploidy, increasing the opportunities for mutational events. A number of antibiotics (mainly bactericidal) cause production of reactive oxygen species (136), which induce the low-fidelity polymerase DinB (PolIV), increasing mutagenesis, as occurs in *E. coli* with beta-lactams; in addition, low concentrations of these drugs induce the RpoS regulon, reducing MutS availability and thus resulting in further mutagenesis and less mismatch repair (137). There is a need for quantifying effective stress levels, occurring in a window of adaptive changes allowing possible evolutionary rescue between no effect and extinction of stressed populations.

Antibiotics as Drivers of Populational Variation

An important evolutionary consequence of antibiotic exposure consists of the changes in the population structure of microbial organisms, including clones (138). The acquisition of AMR occurs in particular clones that are then selected and subsequently compete with and eventually replace others that remain susceptible. Clonal replacement takes place through two main processes (23). The first is exogenous invasion, in which a resistant clone arrives at a particular host, colonizing the skin or mucosal surfaces, eventually increasing its absolute size by antibiotic selection and displacing other susceptible clones of the same or different species, thereby implying local clonal shifts. Exogenous invasion by a resistant clone does not necessarily require antibiotic selection if the clone is well endowed with colonization factors. Invader strains generally succeed when their reproductive numbers exceed that of the background established strain; however, there are scenarios in which the less fit succeed in replacing the previous colonizer (139). The second process leading to clonal replacements is endogenous conversion. As in gene conversion, the term "conversion" in this context means that a successful biological entity already established in a particular environment acquires an adaptive trait present only in part of the analogous entities coexisting in the same setting, even in a transitory manner. In this case, AMR enters into a well-adapted, high-density endogenous clone.

Selection for Resistant Noninheritable Phenotypes

Evolution of inducibility of AR mechanisms. A number of AR mechanisms are inducible, i.e., are expressed at a sufficient level only in the presence of an inducing agent, frequently the antibiotic substrate of the resistance or a related molecule. Classic examples of inducible resistance are inducible penicillinase induction in Gram-positive bacteria such as *Staphylococcus* and *Bacillus* (140) and macrolide resistance in Gram-positive bacteria (141) and *Bacteroidaceae* (142). In general, the inducibility of resistance genes at very low (subinhibitory) concentrations supports the hypothesis that antibiotics in nature act more as highly diluted deterrent "signals" between potentially competing populations than as real killing weapons; that is, they follow the ecological principle of "armament-ornament" duality (143).

The "inducer" effector molecule might be not the antibiotic itself but certain cell metabolites released as a consequence of antibiotic-cell interaction. For instance, the LysR-type transcriptional regulator AmpR activates the expression of chromosomal AmpC beta-lactamase in many proteobacteria in response to changes in peptidoglycan (PG) metabolite levels that

occur during exposure to beta-lactams (144). Two-component regulatory systems (TCS) are involved in a number of AMR-inducing processes, such as VanA operon-mediated vancomycin resistance, which involves the VanS protein detecting the signal produced by glycopeptide action, thereby activating (phosphorylating) VanR, acting on the essential promoter of the Van operon (145). The TCS-mediated processes (and the intensity of induction) might be modulated by other proteins, termed TCS connectors, by affecting the phosphorylation state of the response regulators (146). For instance, the *erm* gene family encodes inducible resistance to macrolides, lincosamides, and streptogramin (MLS) antibiotics by producing enzymes that catalyze *S*-adenosyl-L-methionine-dependent methylation of an adenine residue in the 23S rRNA gene molecule, resulting in the loss of MLS binding to the ribosome. The induction mechanism, provoked by ribosome stalling, involves a change in the hairpin secondary structures of mRNA, allowing the expression of the methylase. In addition to endogenous inducers, ARGs can be induced by exogenous compounds, which is the case for efflux pumps that serve to adapt bacteria to the potential injuries present in their habitat (147), which respond to bile, present in the gut of the colonized host (148), and efflux pumps from environmental pathogens, such as *Stenotrophomonas maltophilia*, whose expression is induced by plant-produced compounds (149).

In general, the mechanisms for the evolution of inducibility are thought to be based on the coordination of economy (fitness), preventing the production (and its consequent cost) of traits that have no function except in the presence of the substrate. However, if exposure to the challenging agent is frequent, a constitutive (constant) expression will spare the costs related to the induction machinery processes, the "costs of phenotypic plasticity" (150). This production acts as in the common good, reducing in some cases the local activity of the drug and facilitating the survival of many inducible cells in the population during early exposure, which are then induced and reach full resistance. The proportion of "constitutive" resistant cooperative mutants for a particular gene (mutation rate plasticity), in relation to the cheater inducible population, might reflect these global adaptive needs. The role of the "regulatory genome," including the quorum-sensing network, is probably critical to understanding the evolution of AMR (151).

Selection of persistence and the evolution of antibiotic resistance. The conceptual differences between resistance, tolerance, and persistence have been analyzed in depth (152). In resistance and tolerance, the entire bacterial population is involved. Persistence is a noninheritable property of a fraction of an otherwise genetically susceptible bacterial population that exhibits phenotypic insusceptibility (persistence) to antibiotics, being able to survive (viable) in the presence of antibiotics at concentrations at which the majority of the population dies off. Stress favors the switch to persistence, which is also frequently related to the random induction of alarmone (p)ppGpp activation (153). Moreover, the persister phenotype frequently offers protection from death from a broad spectrum of unrelated antimicrobial agents (cross-tolerance). The evolutionary importance of this type of "phenotypic selection" is that it might facilitate the generation and ascent of inherited, specific resistance to antibiotics (154), including antibiotic combinations (155), or it might promote the spread of AMR plasmids (156). Persistence ensures viability and hence the ability to evolve but does not necessarily indicate the total absence of antibiotic effects on the cell. Thus, persister variants able to acquire certain replicative abilities in the presence of the antibiotic should be selected with their heritable changes. In summary, there is an epistatic synergistic interaction between resistance and tolerance mutations that has been experimentally observed in strains that have evolved under intermittent antibiotic treatment (157).

An important issue in this respect is how AMR evolves in nongrowing populations. Given that environmental conditions (including antibiotics and other stressors) induce the same set of responses involving similar regulators, all leading to a nonreplicating status, a general core hormetic (dose-dependent) stress response has been proposed (158). A nongrowing status might increase the mutation rate and thereby the selection of mutational traits under antibiotic exposure (159).

The evolution of antibiotic tolerance, either by increasing the drug concentration that the bacteria are able to tolerate or increasing the proportion of tolerant variants, is an

interesting issue that has been scarcely investigated (160). The number of genes involved in bacterial tolerance (the tolerome) is larger than the number of genes identified for the resistome, suggesting that increased tolerance might evolve even faster than AMR (159). Consequently, the question is whether tolerance reduces resistance or favors survival under antibiotic (potentially mutagenic) exposure, thereby increasing AMR.

SELECTION OF ANTIMICROBIAL RESISTANCE

Antibiotic Selective Concentration Gradients

There is a correspondence between antibiotic concentrations and the selection of bacterial genetic variants with various levels of AMR. The number of very low-level resistance mechanisms (many derived from gene mutations providing housekeeping functions) is revealed only at very low antibiotic concentrations, and, in any case, these mechanisms are more numerous than those providing high-level resistance. However, low antibiotic concentrations might still be deleterious for some emergent resistant mutants, resulting in them dying before producing a lineage (161), but that might be mitigated by cell abundance. With strong bottlenecking, strong selection for a few mechanisms is expected to occur (100, 162). Fewer resistant variants are therefore expected to emerge under exposure to high-level antibiotic concentrations; however, those variants would have high-level, highly specific resistance mechanisms.

In the real world, bacterial populations are exposed to antibiotic gradients, the consequence of the molecules' diffusion in a continuous space. When antibiotics are administered to a particular host (such as human patients and livestock), there is a wide set of gradients of antibiotic concentrations in the tissues and mucosal surfaces, and bacteria are subjected to a diversity of concentrations (163, 164). The release of antibiotics in natural ecosystems through wastewater further expands the range of antibiotic concentrations that bacteria can encounter. Each concentration (each point in the gradient) should be able to inhibit the population susceptible to it and to select the organisms able to resist this concentration; however, further up the gradient, these organisms might be inhibited or killed. The selection of a particular variant therefore takes place only in a "window of selection," a process termed concentration-dependent selection (165–167). Concentration gradients create a large "environmental spatial diversity" (168), which, when confronted with the "genetic diversity" of bacterial cells, enables the precise selection of particular variants with even small phenotypic differences, enabling a step-by-step evolution from low- to high-level resistance, favored by gradient shifts (169, 170). Therefore, local antibiotic exposure in compartmentalized spaces might in fact produce a "bunch selection" effect, in which allelic variants of various levels of AMR are selected as a group or cluster in neighboring spaces of the gradient. This spatial proximity and the possible gradient fluctuations facilitate cross-recombination between independently selected variants.

The concentration at which the significant gradient for the antibiotic effect begins to act will depend on the minimum selective concentration, which is much lower than the MIC (171). This minimum selective concentration can be compared with the minimal effective antibiotic concentration (MEAC), the minimum antibiotic concentration able to produce any effect on bacteria (e.g., by acting as a signal and by influencing metabolism) (157, 165). Antibiotic gradients not only vary over time but are frequently embedded in other variable gradients, due, for example, to the presence of other antimicrobials and other selective attractors, producing multivariate extended selection landscapes (172).

Historical Events in Antimicrobial Resistance Selection

The early 20th century (1910 to 1945) saw profound social changes, including troop mobilizations in two world wars, worker and refugee movements, the emergence and development of big pharma, intensive farming, extensive mining, and the growth of the food industry. During this period, the world also endured massive industrial pollution and ecosystem damage, with the colossal mass production and application of synthetic antimicrobial agents in humans and animals. Antibiotics had in fact been employed since the mid-1940s in human and animal medicine in the midst of a massive increase in the production of anti-infectives (173, 174). From the late 1910s to the late 1940s, a plethora of old and

new antibiotic and antiseptic compounds were simultaneously and massively employed in individuals in crowded settings, such as troops in the military, livestock on farms, and patients in hospitals. The anthropogenic use of antimicrobials includes significantly heavy metals, in particular, copper and silver salts but also mercury, tellurite salts, and arsenates (Salvarsan) in 1910. Copper and silver vessels have been employed for at least three thousand years to decontaminate water and food (175). The translucent, white, and colored glazes of ceramic vessels and kitchenware might also release antiseptic concentrations of the lead, cadmium, chromium, and cobalt (176). Interestingly, many of the "modern" plasmids (and transposons) encoding AMR contain determinants encoding heavy metal resistance, leading to the speculation of whether the selection of these replicons predated the current antibiotic-resistant mobilome.

Synthetic dyes and sulfonamides were subsequently introduced for treating infections, followed by penicillin, streptomycin, tetracycline, chloramphenicol, kanamycin, and neomycin (177, 178), all of which selected for organisms carrying genes able to detoxify the various antibiotic agents. In terms of the evolution of AMR, the important fact is that these genes remain present, are mostly intact, and are still prevalent today.

The historical-cumulative effect of antibiotic exposure on microorganisms can be expressed as genetic capitalism, which refers to the capability of organisms to accumulate resistance mechanisms, either via mutational or gene acquisition events, such that the acquisition of a resistance trait facilitates the acquisition of further resistances: the rich tend to become richer (179). Genetic capitalism enlarges the field of selection (through multilateral antibiotic selection) under antibiotic exposure and has likely influenced the increased prevalence of multidrug-resistant (MDR) pathogens and the spread and maintenance of resistance genes among environmental organisms and commensal bacteria, including those of the normal microbiota. Genetic capitalism might work without antibiotic exposure. Bacteria under stress or adaptive need (not only by antimicrobials) can be enriched by evolvability tools, e.g., the acquisition of MGEs, which might serve as sculptors and carriers of AMR complex determinants.

Pharmacodynamics and Selection of Antibiotic Resistance

Do the bactericidal or bacteriostatic effects of antibiotics have any influence on the frequency, spread, and evolution of AMR? Many ARGs in natural populations correspond to bacteriostatic antibiotics, such as tetracycline, chloramphenicol, macrolides-lincosamides, and sulfonamides. Among the bactericidal antimicrobials, only those acting directly on physical cell structures (rather than processes), as antimicrobial peptides, appear to be less prone to contributing to the emergence or selection of resistance genes. The differentiation of antibiotics into bacteriostatic and bactericidal is extremely dependent on human criteria. In addition to pharmacokinetics (available antibiotic in contact with the bacterial cell), numerous factors modulate the lethality of antibiotics, such as cellular responses, the expression of SOS and RpoS systems, the effect of reactive oxygen species, and metabolic and environmentally regulated adaptations (136). Hypothetically, less lethal antibiotics could preserve susceptible populations more than stronger antibiotics; however, high lethality should reduce the cell's possibilities of adapting to the antibiotic challenge.

TOPOLOGY OF EVOLUTIONARY TRAJECTORIES

Trajectories and Fitness Landscapes

In the classic fitness landscape metaphor (Fig. 4) developed by Wright (180), which essentially persists in modern computer-generated landscapes, there is a "horizontal plane" (with different genotypes represented by binary sequences of two types of basic units) and a network of possible mutations between the genotypes forming a hypercube graph. The fitness (reproductive success) of each of these genotypes is represented by a corresponding "height" on the vertical axis. In this plane, the binary (0/1) representation shows the absence or presence of two different alleles of a gene or a particular point mutation. Other "beyond the hypercube" computer landscapes, considering not only binary representations but also 4 (nucleotides) or 20 (amino acids)

alternatives, might produce more realistic landscapes, with higher possibilities of finding trajectories to gain access to fitness peaks (180). How many genotype possibilities are contained in this "soil" plane? In terms of nucleotides, one of the organisms with the smallest genome, the proteobacterium *Nasuia deltocephalinicola* (112,091 nucleotides) can reach $10^{67,430}$ genotypes (180).

Natural selection forces populations to follow evolutionary trajectories along uphill steps of increasing fitness (181, 182). The important issue in the predictability of evolutionary trajectories is when there is only a limited number of trajectories available, travelling from distinct adaptive peaks to reach a final optimal genotypic state (183) (Fig. 4).

In multipeaked fitness landscapes, as in real environments that might be highly variable in both space and time, evolutionary trajectories necessarily should be able to cross valleys, with low fitness and a certain risk of stasis or extinction of the evolutionary objects. It is widely assumed that many, if not most, adaptations are associated with trade-offs, such that changes in traits that increase fitness in some environments or situations are deleterious in other environments or situations (184). For instance, a resistance gene can help the host strain climb high fitness peaks during therapy. In the absence of antibiotic exposure, however, this gene might lead the organism into a valley, resulting from a gene burden for the cell physiology. The changing dynamics of fitness landscapes constitute the main condition of evolutionary changes.

In some cases, survival in valleys might facilitate climbing the next fitness hill. Initially deleterious mutations (sinking the strain in the valley) might serve as gateways for otherwise relatively inaccessible areas of sequence spaces, which might result in positive epistasis with other mutations, thus facilitating uphill trajectories, as observed with TEM-15 beta-lactamase (185). "Long-term advantageous" but at first sight deleterious mutations can be fixed in small populations, and even slightly deleterious ones can also be fixed in relatively large populations (186, 187). Given these potential advantages, sufficiently large bacterial populations can cross fitness valleys, which is probably not the case for small populations (188, 189). It is possible that competition might occur between simple and complex evolutionary trajectories. In rugged landscapes, simple trajectories tend to exploit the immediate easy-to-reach fitness peak. Consequently, however, access to higher peaks might be hampered. In the presence of high population sizes, the fixation of beneficial mutations takes longer, and the genetic diversity of the population is maintained, favoring the collection of adaptive mutants and their interaction, potentiating the population to climb higher peaks by "stochastic tunneling" (190, 191). In any case, we stress here the importance of "abundance"; the organisms presenting greater population abundance have a greater chance of finding effective evolutionary paths to increased resistance.

The standard bidimensional and tridimensional representations of fitness landscapes have contributed to the understanding of evolutionary trajectories. However, these representations are insufficient for imagining extremely complex trajectories crossing deep fitness valleys and spaces when the fitness peaks are spaced far apart. However, imagine smoothing out the creases caused by crumpling a sheet of paper into a ball. The result is a wrinkled texture with "peaks" and "basins" formed by the confluence of creases, which resemble a fitness landscape. These irregularities were (probably more pronounced) in the paper sheet before the ball structure was disturbed. However, the fitness peaks that are distant from each other in the smoothed-out state can be spatially close in the crumpled form (Fig. 4 and 6), meaning that a particular genotype has access to increased fitness in another peak apparently inaccessible in a flat landscape. Complex environments that are more demanding and stressful should produce more peaks and basins, which can be represented by the compressing, squeezing intensity exerted on the crumpled paper ball. Despite the high complexity of the resulting landscape, this "intensity" might be measured by a single global quantity. The evolution of damage in crumpling dynamics can largely be described by a single global quantity: the total length of creases (192). The physics and complexity of crumpled balls have been studied (193) but not their applications in evolutionary biology.

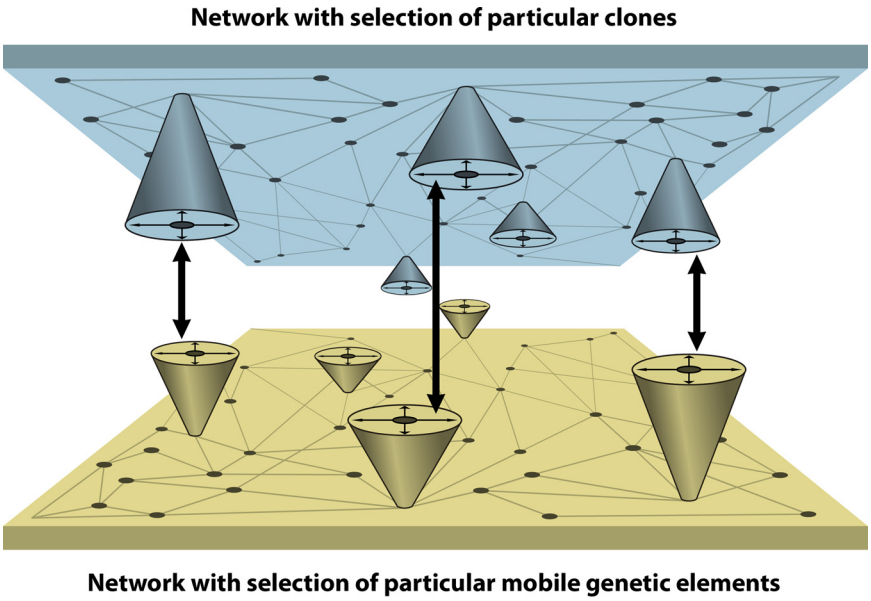


FIG 6 Interactions between evolutionary networks. The top and bottom horizontal fields depict networks where the respective bacterial clones and mobile genetic elements (MGEs) harboring resistance genes evolve independently. In each of the network planes, there are selection events, amplifying the clones or MGEs (cones). Occasionally, a successful plasmid interacts with a successful clone (two-headed arrows), eventually creating a high-risk resistant clone. (Inspired by and adapted from the classic figure by Feil and Spratt [633] with permission of Annual Reviews, Inc.)

Trajectories and Environmental Heterogeneity

A high frequency of random changes in the bacterial genome have consequences for the fitness of bacteria in different environments. In a classic study, individual random insertion mutants of *E. coli* were assayed in four different environments, and it was found that approximately 40% of the insertions yielded different fitness effects in the different environments, showing that genotype-by-environment interactions are common (194). An essential goal of research in antimicrobial resistance is to quantify the risks for AMR of environmental overlapping (195–197). Merging resistome-rich environments provides a wealth of possible new operative material (genes), vehicles (such as MGEs), and genetic partners, able to produce unexpected evolutionary trajectories (198–200).

The influence of "positioning" in bacterial evolution has been well documented in the case of dense, surface-attached, spatially structured bacterial communities (201). Selection of particular variants will occur at some "positions" in the space and not in others. Genetic variants might self-organize in the space, producing an adaptive radiation to occupy neighbor niches (202), eventually leading to a functional division of labor (203). The rates of environmental fluctuation might modulate the level of radiation in novel niches, and competition between variants and the benefits of the "ancestor niche" might act as an attractor limiting diversification. A similar centripetal dynamic occurs in complex communities, where coresident species (and clones) have access to common gene pools, including AMR. However, centrifugal migration should increase the impact of the horizontal transfer of resistance, which would be limited in areas of replication, where vertical transfer predominates.

Local species' coexistence and exclusion within the multiscale and multispecies context within metacommunities should necessarily influence the evolution of AMR, which will occur in spatially close colonization areas. Why are groups of microorganisms spatially linked? We have discussed above coexistence through the sharing of subniches; however, this implies a frequent "sharing of a common goal" (cooperation). Ecologically cohesive populations give rise to genetic exchange communities. Modern metagenomic-bioinformatic techniques, such as high-throughput chromosome conformation capture (3C) technology, might be useful for detecting ensembles if resistance genes hosted by particular bacterial species or groups of species (204, 205) can identify genetic exchange communities. This

important topic is studied by investigating the spatial heterogeneity and co-occurrence patterns of microorganisms in their habitats, including the human mucosa-associated populations (206, 207).

Eco-Evolutionary Spaces of Variation in Chromosomal and Mobile Genes

Gene evolution can be considered a numbers game, depending on the number of gene copies, the gene's long-term stability, the diversity of environments to which the replicon hosting the gene is exposed, and the bacterial host and niche in which it is present. The number of gene copies (such as for a preresistance or resistance gene) determines its evolvability rate, a number that primarily derives from the rate of host replicon replication (bacterial host, mobile genetic element) so that genes from the more abundant and spreading organisms should evolve faster. Given that genes in plasmids multiply in the host cell and, taking advantage of the host replication, might propagate in different hosts (exposed to an expanded variety of environments), plasmid-located genes (including ARGs) should evolve more rapidly than chromosomal genes, as belonging to different units of selection (Fig. 1).

MGEs have another advantage for hosting rapidly evolving genes. The adaptive strategy of chromosomal variation (for instance, in genes encoding the targets of antibiotics) to increase AMR might be considered much riskier in terms of fitness reduction for the bacterial host than for acquiring novel traits by MGEs. Chromosomal genes are frequently inserted into highly regulated interactive biochemical networks that cannot be modified without harm to the system's equilibrium. In addition, the functionality of heterologous chromosomal genes in a particular host is constrained by the compatibility with the host cell's physiology (208). In contrast, foreign genes acquired by HGT, such as ARGs, should in principle be better tolerated, given that they are frequently "decontextualized"; the genes do not belong to the basic network involved in the new host physiology.

Various mechanisms of resistance are accessible by the evolutionary (mutational and recombinational) space of single organisms, such as SHV-type beta-lactamases in *Klebsiella pneumoniae*, which are very close to (and probably originated in) the chromosomal beta-lactamase proteins of this organism (209). However, the beta-lactamases probably only evolved when these SHV enzymes were propagated in plasmids. Certain highly efficient mechanisms of resistance are simply unavailable through chromosomal evolution in the original pathogenic hosts. CTX-M enzymes have not evolved in their original host (*Kluyvera* spp.); the only possibility of acquiring these characteristics has been by HGT when present in organisms such as *K. pneumoniae* or *E. coli*.

EVOLUTIONARY TRAJECTORIES OF ANTIBIOTIC RESISTANCE GENES

The evolution of most ARGs is the evolution of particular changes in gene sequences, resulting in amino acid changes that increase or expand the host organism's fitness when exposed to antimicrobial agents. It is difficult to separate the "resistance gene mutational space" from the "resistance protein space of variation"; however, a correspondence between regions of the resistance gene sequences and the protein sequence spaces is expected (210). Mapping protein sequence space is a complex issue, given that for a protein of length N, the number of amino acid combinations is 20^N. Mutational changes tolerated by the bacterial organism, however, might not necessarily produce a higher fitness phenotype; in many cases, mutations are neutral or "nearly neutral."

Gene evolutionary trajectories are constrained and sometimes facilitated by the genetic code, which translates genetic information in the protein structure and constrains the mutational exploration of the sequence space (211, 212). Expanded codes might increase the number of AMR mutational trajectories (213). In accordance with the error minimization hypothesis, the organization of the pattern of codon assignments is itself the result of natural selection, buffering genomes against the impact of mutations (214, 215). Thus, the number of "functional variant proteins" might be minimal compared with that of all the variant proteins. How large is that minority? Considering that only four amino acids are critical for the interaction between two proteins in *E. coli*, only about 1% are functional, suggesting

context-dependent mechanisms for certain amino acids, which explains why many variants are not observed in nature (216).

Mutational Cost and Compensation: Mutational Robustness

Any mutational deviation influencing the regular optimality of bacterial fitness in relation to a particular environment has potential deleterious consequences, and mutational robustness prevents this risk. The consequences of fitness costs can be expressed as reduced growth, virulence, or transmission. There are many strategies for mutational robustness, which can include the following: (i) gene redundancy, (ii) domain redundancy, (iii) gene overexpression, (iv) presence of genes and pathways with alternative functions, (v) intervention of gene regulatory networks, (vi) reduction in the need for the mutated gene function by reducing the growth rate, (vii) increasing the removal of R-loops produced by translation-transcription conflicts, (viii) focusing on alternative sources of metabolites or energy by moving to a new environment (plasticity), and (ix) the possibility of interactive cooperation with other microorganisms supplying the lost metabolite or function (108, 217–225). Gene functional redundancy refers to genes with partially overlapping functions, in other words, degenerated. In any case, degeneracy is a main contributor to adaptive flexibility and, in general, to functional robustness and evolvability (226).

Mutational fitness costs are not necessarily proportional to the efficiency of mutations in producing resistance. In fact, fitness costs might decrease with increasing AMR (227). The cost of a newly acquired resistance mutation also depends on other mutations in the genome, including other resistance mutations (epigenetic effects), and on the evolutionary history of the organism (228). The damage to bacterial fitness ultimately produced by AMR mutations can be ameliorated by intragenic or extragenic second-site mutations. The more relevant intragenic mutations are those modifying the functional or interactive core of the affected protein, but also "second shell" mutations in neighbor gene domains might have low-level but significant evolutionary effects (229). Although less explored, gene amplification can also contribute to restoring the fitness of antibiotic-resistant populations (230). A final issue concerns the noninherited compensation of the effect of AMR. One example of this possibility is the increased expression of a gene that can compensate for the lack of activity in a gene that mutates to acquire resistance; this situation can also be due to overexpression due to changes in regulation (221).

Mutation Founder and Competition Effects

If a resistance gene is acquired and spreads quickly in the population, the chances of acquiring a new resistance gene conferring the same phenotype might be low, a situation termed a founder effect, which might explain the low number of different resistance genes acquired by human pathogens compared with the high number of potential resistance genes that can be found in any analyzed microbiome (30). Mutants with higher fitness costs or that are less able to compensate for these costs will disappear more rapidly in the absence of selection than the fitter mutants. In addition, mutants that are fitter in the presence of antibiotics will displace the less fit ones under these conditions, i.e., in treated patients. The latter situation occurs in populations in which the mutation supply is high (i.e., large populations and/or with increased mutation rates). This leads to competition between cells with distinct AMR mutations, a concept known in classic evolutionary theory as clonal interference (231). Clonal interference has been shown to influence the compensation and reversal of AMR (232).

Epigenetic Epistasis Shaping Mutational Trajectories

Genes encoding AMR are most frequently part of a network of interactivity with other genes and genetic contexts. The main genetic context providing flexibility is the rest of the gene sequence; however, other neighbor and eventually coregulated genes, the genes of the genome of the bacterial host, and probably the genes of other functionally linked communities of microorganisms successively influence the expression and consequently the evolutionary trajectories of ARGs (233, 234). The study of all these functional gene-gene constellation interactions is the field of epigenetics,

referring to heritable (reproducible) changes in gene function that cannot be explained by mutations in DNA sequence, studying the "over-the-gene" events in modifying gene function (235–237). Certainly, ARG evolution and evolution in general cannot be explained or predicted without understanding how gene interactions shape adaptive possibilities (238). Environmental fluctuation and range expansion (the organism's progeny is exposed to different environments following population expansion) might increase epistatic effects and adaptability, accelerating evolution (239, 240). Based on *in vitro* experiments, it has been proposed that a single new beneficial mutation might interact with ensembles ("blocks") of other potential beneficial mutations with positive or negative mutational sign effect, eventually resulting in the selection of new blocks and the whole evolutionary trajectory (241). A number of adaptations, including the case of AMR, are associated with epistatic trade-offs, such that changes in traits that increase fitness in some environments or situations are deleterious in certain other environments or situations (242). In general, epistatic events are neutral or negative at early stages of a trajectory and more beneficial at later stages (185).

Such epistatic interactions not only occur when genes are mutated but could also be due to variation in gene expression, including among isogenic individuals in a controlled environment (243). Early mutations in global transcriptional regulators, favored by environmental changes, might cause extensive changes in the expression of a multiplicity of genes, which will be subjected not only to positive selection but also to negative epistatic interactions (244). Stochastic variation in the expression of sets of genes is expected to occur, even in isogenic populations, due to factors that transiently modify the gene function, including DNA methylation, covalent modification of DNA binding proteins, noncoding DNA, and RNA splicing factors. These factors produce epigenetic variation by influencing stochastic fluctuations in cellular components and consequently might affect the expression of AMR traits. These effects are probably more effective in high-order epistasis (in which the effect of a mutation is influenced by two or more other mutations), which facilitates the accessibility of evolutionary trajectories (245). However, other studies have indicated that epistasis remains rare even when up to four chromosomal mutations are combined (246).

Mutational Paths in Genes Involved in Antimicrobial Resistance

There are a number of mutational paths in genes that already provide AMR phenotypes, leading to new variants, either increasing the ability to resist at higher concentrations of a particular antimicrobial agent, extending the spectrum of inactivation to other antibiotics, reducing the killing (bactericidal) effect of drugs, or reducing the fitness costs of these genes' expression. These paths (or at least those that are able to be detected) appear to be relatively limited in number and do not necessarily produce the fittest theoretically possible phenotypes (in terms of selectable AMR), except for those able to become more abundant in general (247).

Variant penicillin-binding protein-mediated resistance. The paradigmatic case is beta-lactam resistance in *Streptococcus pneumoniae*. When susceptible bacteria are exposed to increasing concentrations of penicillin, the acquisition of mutations by the penicillin-binding proteins (PBPs) PBP2x and PBP2b, the main resistance determinants, is extremely ineffective in determining clinical AMR. Specifically, when the antibiotic target protein is functionally linked in a complex interplay with other proteins (in this case to ensure construction of the cell wall), the maintenance of function requires other cascade changes, which are very difficult to achieve by simple evolutionary events. For instance, changes in PBP2x and PBP2b are relevant only if PBP1 is also altered (248). High resistance to penicillins occurs only if several PBPs (e.g., PBP2x, PBP2a, and PBP1) are altered at the same time, and also genes such as those encoding MurM and MurN, involved in the supply of substrate molecules to the PBPs, are involved. The recruitment of mutations in PBPs and MurM/N proteins occurs efficiently by successive recombination events, following horizontal acquisition of chromosomal fragments containing natural or mutant resistant PBPs from neighboring species, such as *Streptococcus oralis* (249). There does, however, appear to be a limit to the

incremental acquisition of variant or mutant PBPs as the biological cost increases with the number of acquired resistant PBP alleles (e.g., in competition experiments with their susceptible ancestor to colonize the respiratory tract) (250).

Mutational paths in variant DNA topoisomerases. Site-specific mutations in a number of target genes (quinolone resistance-determining region mutations) account for incremental resistance to fluoroquinolones. *In vitro* serial passage evolution experiments in various organisms indicate that stepwise access to high-level resistance can be achieved with a (relatively) nonrandomly ordered sequential fixation of mutations, following pervasive mutational interactions. In *S. pneumoniae*, mutations in the ParC subunit of DNA topoisomerase IV (a primary target of fluoroquinolones) should be acquired first, followed by further mutations in the DNA gyrase A subunit, resulting in the formation of a high-resistance phenotype (251). Next, the acquisition of a new mutation in ParE increases the fluoroquinolones' MICs (252). However, this canonical evolutionary path is not universal. Experimental evolution performed in parallel with several lineages derived from a single ancestor pointed to the possibility of different paths, and the order of mutations can differ in other organisms; GyrB primary mutations can occur more frequently in other organisms (e.g., *Helicobacter pylori* and *Mycobacterium tuberculosis*). As in the case of variant PBPs and beta-lactams, the acquisition of mutations in topoisomerases (eventually altering DNA supercoiling) might influence the strain's fitness and consequently its selectability and potential evolutionary trajectories.

Target gene conversion. In the case of homologous repeated genetic sequences of a target gene in a single cell, the mutation acquired in a copy (generally producing low-level resistance) might easily be reproduced by intragenomic recombination in the other copies (providing a high-resistance phenotype). This phenomenon is known as "gene conversion," and it ensures the nonreciprocal transfer of information between homologous sequences inside the same genome. For instance, single-mutation rRNAs easily produce AMR to aminoglycosides (253). In the case of linezolid (oxazolidinones), the G2576T resistance mutation in domain V of 23S rRNA occurring in a single copy (very low-level resistance) propagates in the other copies by RecA-dependent gene conversion, facilitating access to high-level resistance (254). Gene conversion might also contribute to restoration (repair) by recombination of the wild sequence of the susceptible phenotype and, in general, to the concerted evolution of multigene families (255), which would be an easy method for reverting resistance and compensating its costs.

Mutational paths in evolution of detoxifying enzymes. Weinreich et al. focused on the evolutionary possibilities of TEM beta-lactamase in *E. coli* (256) employing a model that included five-point mutations in the basic TEM-1 allele, which is able to move the resistance phenotype from aminopenicillin-only to high cefotaxime resistance. Evolution to cefotaxime resistance might follow any of the 120 theoretical mutational trajectories linking these alleles. It has been demonstrated that most of these trajectories (85%; 102 trajectories) are inaccessible to Darwinian selection and that many of the remaining trajectories have a negligible likelihood of being traversed, such as contained fitness reduction and neutral steps, including sign-epistatic interactions (mutation providing resistance to antibiotic A increases susceptibility for antibiotic B), resulting in significantly reduced chances of being followed by natural selection (256, 257). To overcome such antagonistic pleiotropy, new ("modulatory") mutations are required (258). The mutated protein should be not only active but also sufficiently stable, and a number of apparently neutral mutations, including suppressor mutations, are required for reorganizing the topology once "advantageous mutations" have been achieved (i.e., stabilizing mutations) (259).

Inactivating enzymes: beta-lactamases and aminoglycosides. Evolutionary biology often assumes that for any protein, natural selection explores all adaptive options for achieving optimal efficiency. Although the diversity of TEM enzymes is high (currently more than 225 variants), affecting up to 32% of amino acid positions, several authors have demonstrated that only 13 to 16% of the positions in TEM-1 beta-lactamase do not tolerate substitutions (the enzyme's core), without critically or drastically reducing

hydrolytic activity. More diversity should therefore be present in the real world, which is not the case. The reason for this difference is that many changes have a neutral effect. It also has been shown that the neutrality of these changes is itself conditional on the selection strength; i.e., under weak selection (for instance, low ampicillin concentrations), the vast majority of mutations are statistically neutral; under strong selection (high ampicillin concentrations), however, the enzyme's overall fitness cost and the proportion of variant alleles is dramatically increased (260). The concepts of strong selective pressure, fitness, and global protein stability are closely related (261). Mutations influencing the beta-lactamase omega loop, which are found in oxyimino-cephalosporin-resistant variants, reduce enzymatic stability in TEM (261) and CTX-M (262) beta-lactamases. These compensations influencing the enzyme's stability might allow the buildup of strong dependencies among mutations. Weinreich et al. demonstrated that only 15% of all possible mutational pathways convert TEM-1 in an effective cefotaxime-hydrolyzing enzyme, with a predetermined fixed order in the incorporation of each mutation (263). The improvement in MIC provided by the enzyme is not the only evolutionary goal for antibiotic-inactivating proteins such as TEM enzymes. Protein stability is also an important driver; highly stabilized variants of TEM-1 beta-lactamase exhibit selective rigidification of the enzyme's scaffold, while the active-site loops maintain their conformational plasticity (264). Novais et al. studied the fitness landscape in CTX-M mutants, identified those positions under positive selection, and constructed all their mutational combinations (265). Only a few trajectories were possible from an ancestor CTX-M-3 until a more efficient enzyme for hydrolyzing ceftazidime was reached (CTX-M-58). Nevertheless, the authors observed that the number of evolutionary trajectories could be increased if the environment fluctuated between two antibiotics, such as ceftazidime and cefotaxime, so that antibiotics are both selectors and accelerators of variant diversity (266). A mutational founder effect might diversify the evolutionary trajectories (that become incompatible). For instance, the CTX-M-3 mutations P167S/T and D240G were antagonistic, being both able to give rise to separate trajectories to improve the efficiency of the enzyme. In TEM or CTX enzymes, antagonistic pleiotropy between different phenotypes occurs, particularly between enzymes more efficient in inactivating cephalosporins and those resistant to beta-lactamase inhibitors (256).

If the initial mutation determines the evolutionary trajectory, are there factors that affect the choice and selection of one or another trajectory or that depend only on random events? The fastest trajectory in the fitness landscape depends on the relative magnitude of the mutation rate and population size (267, 268). In small populations with a low mutation rate, the shorter trajectories to reach the fitness peak occur, such as P167S/T mutation in CTX-M following a "survival of the fittest" dynamics. In contrast, in large populations with higher mutation rates, the most successful strategy follows slower trajectories, such as the D240G mutation, a "survival of the flattest" dynamics (265, 269), because under these conditions the fittest organisms are those showing the greatest robustness against the deleterious mutations (270). According to clinical evidence, the survival of the flattest in AMR is generally the most successful strategy, because the antibiotic bottlenecks select microorganisms with high mutation rates (110).

The case of aminoglycoside-inactivating enzymes, adenyltransferases, phosphotransferases, and acetyltransferases, provides a different example of available evolutionary trajectories. Most of these enzymes probably derive from actinomycete ancestors, and horizontal transfer after gene capture in integrons, transposition, and conjugation has possibly contributed to allelic diversification (271–273). In contrast to the case of beta-lactamases, no mutational evolution of the first detected classic enzymes has apparently occurred under aminoglycoside clinical exposure. Hypothetically, several of these enzymes could have slightly ameliorated their abilities to inactivate recent aminoglycosides.

Mutational paths in efflux pumps. Gene-dosing and higher transcription effects increase the efficiency of chromosomally encoded MDR efflux pumps; high-level expression can be achieved through mutation in their regulatory elements. Evolution to efflux

pump overexpression has actually been observed under experimental evolution conditions (274). The interplay between intrinsic and acquired resistance to quinolones has been shown in *Stenotrophomonas maltophilia* and in other clinical resistant isolates evolving under antibiotic treatment (274, 275). Unlike other resistance determinants, MDR efflux pumps are nonspecific; each independent efflux pump can extrude a variety of antimicrobial compounds belonging to different structural families. In this situation, improving the affinity for one compound might reduce the affinity for other substrates, as described in the case of AcrB (276). A number of examples show that AMR can be acquired by modifying the efflux pump structure (277). However, nearly all studies on resistance and MDR efflux pumps have focused on the overexpression of these resistance determinants, which increased resistance to every toxic compound extruded.

Orthogonality Influencing the Acquisition of Foreign Antibiotic Resistance Genes

Orthogonality is a term borrowed from vector theory in mathematics and widely employed in synthetic biology and computational sciences in systems theory. Orthogonality implies a factual independence between otherwise coexisting systems (278). To be functionally active and not impose fitness costs, a resistance gene (function) should not interfere (should be orthogonal) with the ensemble of genes (functions) of the receptor organism. Full orthogonality is, however, unrealistic, given that the incoming gene necessarily competes with the cell's replication and translation machinery, and the resistance function should be expressed in interaction with the cellular structures. There is a paradox to be considered here: are resistance genes from distant organisms better tolerated, as they are decontextualized, than resistance genes from closer lineages?

Codon usage compatibility between foreign genes and recipient genomes is an important prerequisite for assessing the selective advantage of imported functions and the associated fitness and therefore to increase the likelihood of fixing genes acquired via HGT events (279). However, this cost can be minimized both by in *cis* changes in the acquired gene promoter and in *trans* changes in the host genome, without introducing mutational changes in the ARGs (280). Ribosomal mutations might allow the efficient expression of exogenous genes that are nonoptimal for the tRNA repertoire of the new host (281). There are many decontextualized resistance genes. It has been reported that directional selection on a highly constrained gene previously under strong stabilizing selection was more efficient when it was embedded within a network of partners under relaxed stabilizing selection pressure (282). The ensemble of the genes in a genome (from core genome to pangenome) constitutes something like an integrated ecosystem, the functions of each gene contributing to the formation of an "environment" where the functions of all others should be accurately incorporated in a common, robust ensemble. Gene variation, or foreign gene acquisition required for survival in the case of AMR, is always a stress situation forcing reshaping of evolutionary trajectories to minimize risks of extinction.

MOBILE GENETIC ELEMENTS AND RESISTANCE TRAJECTORIES

MGEs of prokaryotes can be defined as any type of DNA coding for proteins that mediate the movement of DNA either within the cell genome (intracellular mobility) or between bacterial cells (intercellular mobility). Most MGEs have been classically categorized in terms of their basic genetic content, mechanistic transfer properties, or regulatory aspects; however, the categorization of MGEs is difficult ontologically (and thus taxonomically), because the frequent modular exchange of fragments between elements often results in mosaic entities or genetic configurations with distinct functional properties (283–285). The total pool of MGEs, in either cells, populations, species, or multispecies genetic exchange communities, constitutes the mobilome (286). The ecological context appears to determine the abundance and diversity of mobilomes as reflected by MGE enrichment in the gut, oral microbiomes, and particular taxa (287). Such robustness indicates that contemporary MGEs/mobilomes were not born with AMR but that their current abundance, diversity, and complexity are the result of a cumulative series of anthropogenic interventions, a "history of significant events" that continuously shape the evolutionary paths and trajectories of AMR.

The characteristics of mobile genetic elements associated with AMR in main pathogenic organisms have been superbly reviewed previously (288). In this section, we focus more on the ecology and evolvability of MGEs, which have a major impact on the evolution of AMR. Remarkable gene recruitment systems, such as integrons, have been discussed elsewhere (289) and are analyzed here in the context of the MGEs in which they are usually embedded.

Ecology and Evolution of Mobile Genetic Elements

Plasmids. The term plasmid was first introduced by Joshua Lederberg in 1952 to define any extrachromosomal hereditary determinant (290). The demonstration of transferability of AMR phenotypes (alone or in combination) in isolates from epidemics caused by multiresistant *Shigella flexneri* in Japan in the 1950s (291), from *Salmonella* on English farms, and from *Staphylococcus aureus* in European and Australian hospitals in the 1960s led to the landmark discoveries of non-Mendelian infective heredity. Early studies also highlighted the plasmids' ability to cross species barriers, generate novel entities resulting from recombination events, and increase the copy number (and thus the mutation rate) after gene acquisition, making them unique among all the MGEs described to date (291, 292). The biology and epidemiology of plasmids have been extensively (and increasingly) analyzed since their first description (288, 293, 294). However, the role of plasmids in the robustness and evolvability of bacterial populations has been poorly addressed.

Plasmid categorization is based on the diversity of replication (295, 296) and conjugation machineries (297–302) enabling the application of a common nomenclature that can help track ARG propagation and analyze the epidemiological and biological features of various families over decades. A comprehensive phylogenomic analysis based on pairwise identity of the 10,000 plasmids available in public databases demonstrates how plasmids cluster in coherent genomic groups called plasmid taxonomic units (PTUs), which are similar in concept to bacterial species by the analogy of PTUs with bacterial operational taxonomic units (303). This approach provides a more robust plasmid classification (PTUs are poorly correlated with "classical" incompatibility or mobility families), revealing a gradient of host ranges for different PTUs (not all plasmids are equally involved in HGT and therefore have differing effects on the propagation of adaptive features). This issue has been widely analyzed but poorly addressed in the literature because the host range has been based on very few plasmid representatives.

More than half of the PTUs are associated with *Enterobacterales*, *Bacillales*, and *Lactobacillales*, which reflects the predominance of plasmids in the gut and oral microbiomes of humans and animals (302, 304), and are thus involved in AMR. A gradient of host ranges for different PTUs has been inferred from comprehensive genome databases, with the number of mobilizable and conjugative plasmids able to propagate between species of different bacterial genera and families being higher than that of plasmids able to move between orders (as IncL/M, IncN1, IncW, IncHI2, and IncX1) and classes. Epidemiological data complement (and confirm) the heterogeneity of plasmidomes in bacterial populations, from the species to the microbiome level (23, 297, 304, 305). Maintenance of plasmid heterogeneity enables a rapid response to antibiotic challenges in connected environments through broad-host-range plasmids that trigger ARG propagation between host-adapted bacterial populations (306).

In principle, plasmids impose a fitness cost on the cells where they are located. This fitness cost derives from the cellular maintenance, transcription, and translation of plasmid genes, from the interference between chromosomal and plasmid regulators, and from the fitness-lowering effects of plasmid-encoded proteins (307, 308). The generation and maintenance of adaptive plasmid variants have been explained by compensatory evolution to ameliorate plasmid cost (309), which involves chromosomal or plasmid mutations, the transport of partitioning genes or toxin-antitoxin system genes that directly enhance plasmid stability (310), enhanced infectivity, epistasis between plasmids that often coinfect the bacterial cell (311), and source-sink dynamics in multispecies populations (312). Mutations leading to a reduction in plasmid fitness costs

tend to be based on the chromosome if vertical transmission of the plasmid predominates over horizontal transmission. Thus, infectious transmission and compensatory evolution might be competing evolutionary trajectories (313).

One remarkable feature of plasmids is that they typically are kept, on average, at more than one copy per bacterial chromosome, which is particularly true for small, multicopy plasmids that have been shown to accelerate the evolution of AMR by increasing the rate at which beneficial mutations are acquired (124). When new mutations appear in multicopy plasmids, the mutations coexist with their ancestral allele during a number of plasmid generations, which are proportional to the plasmid copy number. This coexistence allows plasmids to provide simultaneous resistance to different antibiotics of the same family, overcoming the restraints imposed by trade-offs in the evolution of antimicrobial resistance genes (314). These features highlight multicopy plasmids as important catalysts of bacterial evolution. An increase in the copy number of conjugative plasmids can occur in the presence of antibiotics to enable gene-dosing effects and to facilitate the acquisition of a costly phenotype in heterologous hosts (315). Comparative genomics of available plasmids help infer subsets of variants that would be adaptive for evolutionary lineages (316). However, the evolutionary trajectories vary among different plasmid categories and plasmidomes, ranging from highly conserved backbones, such as plasmids W, C (formerly A/C), and P1 (317–320), to highly variable subtypes within classical F, I, and X families (321–323).

Antibiotic resistance genes located in plasmids are embedded in other MGEs inserted in the variable region of the plasmid genome, often clustered in multiresistance regions (324). A dense network of extensive plasmid exchange involving genes, MGEs, or chromosomal regions facilitates the adaptation and evolvability of both plasmids and bacterial host populations. As a first possibility, identical genes/MGEs can be captured by various PTUs available in the ecosystem, which can occur by recombination between plasmids or by independent acquisitions from common or different sources. Second, plasmids can recombine, yielding multiple replicons that enable plasmids to replicate in different hosts. Multireplicons occur in the F plasmids in *E. coli*, the nonmobilizable plasmids of *Neisseria gonorrhoeae*, the Inc18 chimeras, and the pheromone-responsive plasmids and RepAN plasmids in enterococci (297, 298). Third, plasmids can mobilize chromosomal regions or elements carrying ARGs and/or virulence factors in *trans*. IncC plasmids of proteobacteria are able to mobilize *Salmonella* and *Proteus* genomic islands (GIs; in this case, SGI1PGI1 elements) and *Vibrio* MDR GIs to *Salmonella*, *Proteae*, *Vibrio*, and *Shewanella* (325–327); IncF plasmids from *E. coli* mobilize high-pathogenicity islands (328); and Inc18 plasmids from *Enterococcus faecalis* are associated with the transfer of large chromosomal regions (329).

Transposable elements. Transposable elements (TEs) are frequently found in plasmids, integrative-conjugative elements (ICEs), bacteriophages, and chromosomes and can transfer between hosts by moving from chromosomal sites to MGEs and vice versa. TE activity constitutes one of the more important forces that affect the evolutionary trajectories of antibiotic/xenobiotic resistance in human and animal pathogens, as well as the trajectories of other MGEs and bacteria. Despite the ubiquity and diversity of TEs (330), the number of different chemical mechanisms employed in TE movement is surprisingly limited, with many divergent TEs sharing similar mechanisms. Nonrandom distribution is a common attribute of TE insertions; however, target site preference for insertion site and transposon immunity varies among TEs, which, in addition to natural selection, determines the distribution of various TE entities and thus their dissemination highways and consequently the spread of ARGs and other adaptive traits. TE self-regulation modulates the extent of damage in the host, with low activity under normal circumstances and activation under stress, which could ensure survival in offspring. Categorization of transposable elements has been based on differing criteria, mainly the diversity of the transposases (Tpases) and the ability to self-mobilize (331). However, borders between TEs are unclear, and there has been an increasing number of reported elements involved in AMR that do not fit into traditional classifications (331, 332).

Insertion sequences. Insertion sequences (ISs) are the simplest autonomous MGEs in bacteria, comprising only one or two proteins needed for their own transposition. In addition to the classical IS model, this category currently includes a variety of IS-related TEs that share various levels of similarity with ISs, all widely distributed and associated with AMR. Nonclassical ISs or "IS-related TEs" comprise self-transferable and nontransferable elements, strongly influencing the trajectories of other MGEs and bacteria, favoring both the acquisition of resistance traits and constraints for the loss of genetic identity of the bacterial organism, maintaining bacterial "evolution on a leash." The analysis of available genomes and metagenomes shows a limited distribution of most IS families among prokaryotes, with an overrepresentation of ISs among certain phyla, genera, and species (287, 333), which is probably associated with the exposure of such bacteria to variable, stressful, and new environments. IS insertions can lead to the capture of ARGs in particular bacterial genomes. A few major IS groups are predominantly involved in the capture or mobilization of ARGs in *Enterobacteriaceae*, staphylococci, streptococci, and enterococci as the members of the families IS*91* (ISCR), IS*6*/IS*26* (IS*26*, IS*257*, and IS*1216*), IS*4* (IS*10* and IS*50*), IS*110* (IS*ECp*1), and IS*1111* (IS*5*), which is probably amplified by HGT events, IS*6*/IS*26* (IS*26*, IS*257*, and IS*1216*), and IS*ECp1* (334–338).

IS insertions can change the antibiotic susceptibility phenotypes toward either resistance or hypersensitivity by modifying the expression of antibiotic uptake determinants, transport processing, target sites, regulatory pathways, and efflux systems, eventually silencing genes/elements (116). At the genome level, interactions between IS elements result in the generation of composite transposons or genome deletions involving ARGs. In addition to the classical examples of composite transposons involving members of the IS*4* (Tn*5*, IS*50*-Km-ble-str; Tn*10*, IS*10*-tet), IS*1* (Tn*9*), or IS*6* family (IS*26*, IS*257*, and IS*1216*), a plethora of possible transposons can be generated using subrogated ISs or subrogated ends (332). However, self-mobilization of these IS derivatives is influenced by the Tpase type and its orientation. The need to differentiate between mobile and nonmobile TEs (TEs versus "pseudotransposons") has been suggested (285). An important feature of IS-TE derivatives is their ability to provide a scaffold for recruiting new genes (335, 336), which can result in novel mobile composite platform variants (339) and select lineage-specific plasmid variants (340). Both insertions and deletions in the genomes where ISs reside are derived from "local hopping" and transposon immunity (341). Recent studies using *E. coli* as the targeted species revealed that IS insertions occur 10-fold more frequently than IS-induced deletion events, even though deletions can vary under or in the absence of selection, implying that the genome tends to shrink without selective pressure (341, 342). Several explanations for IS dynamics using theoretical models have been offered (343–345).

ISs and IS-derived elements are themselves subjected to evolution, and their dissemination and maintenance have been analyzed (346). Transposition bursts are often interpreted as stress responses to environmental changes; however, the accumulation of stress events and elements would lead to unbearable fitness costs and possible extinction of hypertransposed populations following Muller¨s ratchet-like processes, a type of evolutionary fatigue (347–349). Transposition bursts occasionally occur in the apparent absence of stress, ensuring the persistence of ISs in those populations (350, 351). Maintenance and multiplicity of adaptive IS variants can be explained by three complementary hypotheses, focusing on IS selfishness (selfish DNA hypothesis), IS adaptive benefits (adaptive hypothesis), and IS adaptive neutrality (neutral hypothesis).

Main transposon families influencing trajectories. Tn*3* family transposons, classically known as class II transposons, are unitary noncomposite platforms that transpose by a replicative pathway, forming an intermediate cointegrate of donor and target molecules that are fused by directly repeated transposon copies. Most are autonomous elements with a complete transposition machinery that mobilizes the element in *cis*. However, a few Tn*3* composite transposons, pseudotransposons, and nonautonomous elements have also been described. Tn*3* elements display transposon immunity, which precludes transposition of more than one copy of the element into a single replicon

(352). The disparate phylogenies of the transposition and resolvase modules reflect a long coevolution that has resulted in a plethora of Tn*3* elements, typically classified according to the TnpA/IRs (inverted repeats) in large clusters that group TEs from different taxonomic groups, reflecting the general impact of HGT in MGE evolution and explaining the coevolution of TnpA and IRs to maintain specific and functional interactions between genetically connected hosts. Four large Tn*3* clusters are of special relevance in hosting AMR genes, namely, Tn*4430*, Tn*5393*, Tn*21*-mercury transposons, and Tn*3*. Tn*3*, which carries bla_{TEM}, was the first transposon described (originally named TnA) (353, 354) and was already widespread in early plasmids of various incompatibility groups (355). Mercury transposons have long been considered the flagship of AMR, because of the association of Tn*21* with class 1 integrons and other composite multiresistance platforms in early MDR isolates from the 1950s (356). More recent studies have demonstrated a large diversity of mercury TEs in early AMR plasmids of human and environmental isolates, probably selected by the wide and intensive use of mercury in the early part of the 20th century. These transposons would have subsequently and independently acquired class 1 integrons (357). Emblematic examples of Tn*3* mercury members include Tn*21*, Tn*1696*, Tn*501*, and Tn*6182*, all globally distributed in epidemic plasmids or embedded within resistance islands (358–360). Tn*4430* includes TEs widely spread in the staphylococci and/or enterococci, Tn*917* (*ermAB*, encoding erythromycin), Tn*551* (*bla*, encoding beta-lactamase), and Tn*1546* (*vanA*, encoding high-level resistance to glycopeptides). Another group is represented by Tn*5393* (*strAB*), present in all plasmids recovered in the 1950s and clustering other similarly cryptic TEs such as Tn*5403* and Tn*3434*, initially found in the environment and now increasingly associated with mobile composite elements, including the bla_{KPC} and bla_{NDM} AMR genes (361, 362). This group also helps other MGEs; indels and rearrangements are frequent and appear in both contemporary and early plasmids. Composite elements including Tn*3* are apparently exceedingly rare, because transposition immunity precludes transposition of more than one copy of the element into a single replicon. Pseudotransposons and nonautonomous elements related to Tn*3* have been described.

The Tn*7* superfamily comprises unusual, highly sophisticated, and extremely efficient MGEs, which are characterized by their transposition machinery and by displaying, in addition to Tn*3*, transposon immunity (363). Tn*7* frequently targets an *att*Tn*7* chromosomal site (*glmS* gene), an essential gene conserved in highly divergent bacteria. This propagation occurs in a neutral manner and leads to the successful propagation of adaptive traits by vertical transmission. Tn*7* also targets conjugative plasmids and bacteriophages at a low frequency. There are strategies that relax the target specificity, as well as alternative target locations, including interactions with other MGEs, such as mobile cassettes and genomic islands (363, 364). Remarkably, these elements are the main vehicles of class 1 (Tn*402*) and class 2 (Tn*7*) integrons (289, 365, 366). According to the phylogeny of the transposases, Tn*7* elements are classified into three groups: Tn*7*, Tn*5053*/Tn*402*, and Tn*552*, each with a GC content that reflects the preferred bacterial host and thus an ancestral adaptation to distinct prokaryotic groups (364). There has been an increasingly large number of reported Tn*7* variants carrying genes coding for resistance to antibiotics (embedded in class 2 integrons, genomic islands, and IS-related TEs), heavy metals (operons or clusters associated with silver, copper, and chromate resistance), and CRISPR or RM systems, among many other adaptive traits (364, 367), prompted by IS-mediated homologous recombination.

Tn*5053*/Tn*402*-like transposons (TniABQR) have target preference for the *res* site of plasmids and TEs of the Tn*21* subfamily and therefore are known as "*res* hunters." Resolvases (*res*) function to resolve plasmid dimers following plasmid replication. Tn*5053* transposons are predominant in disparate environmental settings and occasionally in clinical isolates of *Pseudomonas* (e.g., Tn*502* and Tn*503*); however, Tn*402* elements are distributed in many prokaryotic groups associated with various hosts. A plethora of Tn*402*-like transposons have been reported, including variants with defective tni_{Tn402}, class 1 integrons, and hybrids

of Tn*7* and Tn*3* (Tn*5053*/Tn*402* and Tn*21*/Tn*501*), which would have spread via HGT and recombination with many different MGEs (368).

Tn*552*-like elements encode the beta-lactamase genes of staphylococci from their early spread after the penicillins' therapeutic introduction. These elements are extremely frequent in multiresistance plasmids typically inserted within the *res* site of the plasmid's resolution system. In many cases, genetic rearrangements are evident within or in the vicinity of these elements, presumably mediated by interactions between the transposon and plasmid resolution systems and repeated transposition events into the elements.

Nonautonomous transposable elements. Nonautonomous TEs are fully dependent on *trans*-acting compatible transposases encoded by related functional (autonomous) TEs and include small (generally less than 300 bp) elements, such as miniature inverted-repeat transposable elements (MITEs) and mobile cassettes, whose transposition can be catalyzed in *trans* by a transposase of a related IS (369). MITEs greatly contribute to the spread of AMR from environmental species into *Acinetobacter*, *Enterobacteriaceae*, and *Aeromonas*.

MITEs and repetitive extragenic palindromic elements are small, nonautonomous IS derivatives whose transposition can be catalyzed in *trans* by a transposase of a related IS (332, 369). These elements are represented throughout the microbial world, indicating an ancestral origin for these sequences. A linear correlation between IS and MITE abundance has been observed, such as the conserved 439-bp MITE-like structures flanking integrons found in *Acinetobacter* species of disparate origins that facilitate the acquisition and spread of various beta-lactamases (370), the integron mobilization units carrying bla_{GES-5} located on plasmids of *Enterobacter cloacae*, and others found in plasmids or in either *Enterobacterales* or *Acinetobacter* (371). Tn*3*-derived inverted-repeat mobile elements are specialized MITEs (372, 373) which can regulate the expression of genes by insertion within protein coding sequences and are responsible for the mobility of antibiotic-resistant class 1 integrons located in both plasmids and chromosomes (370, 371). Different IS families show target specificity for repetitive extragenic palindromic sequences (IS*3*, IS*110*, IS*4*, IS*256*, and IS*5*), which is not surprising given that the features of the DNA target and of the transposase domain responsible for target choice are not included in the criteria for defining IS families.

Genomic islands. Genomic islands (GIs) are large, genomic regions of variable size (4.5 to 600 kb) characterized by the presence of modules that enable the integration, excision, and transfer of the element, although the regulation of these states varies greatly among elements. GIs comprise a plethora of the "island family" composite platforms including ICEs, pathogenicity islands, resistance islands, symbiosis islands, integrating plasmids, and prophages. Most prokaryotic groups have different types of GIs (374). GIs enable the adaptation of bacteria to environments, often in quantum leaps, allowing bacteria to gain large numbers of genes related to complex adaptive functions in a single step, thereby conferring evolutionary advantages; examples include GIs of staphylococci (SaPIs and SCCmec), *Vibrio cholerae* (sxt/R391 and other GIs), *Salmonella* (SGIs), *Acinetobacter* (AcRo), and *Proteae* (PGIs), which can be mobilized by plasmids or phages (374). Most of the available information on ICEs comes from comparative genomic analyses revealing gene content, functionalities, and evolutionary history (375). Certain ICE families have been characterized in detail, especially those associated with AMR, such as sxt/R391 (MPF_F type), Tn*916* and ICEBs1 (MPF_{FA}), and CTnDOT (MPF_B). These cases show that ICEs have greatly influenced the fitness of bacterial lineages (376).

The different requirements for the integrated and excised forms of GIs/ICEs now suggest the inability to coexist in the same cell and have led to the hypothesis that most ICE systems go through a bistable activation state, followed by ICE excision of a dedicated subpopulation and possibly by a specific transfer competence development program (377, 378). The bistability hypothesis helps to understand the lifestyle of ICEs and the selective forces behind their vertical and horizontal transmission modes. The small size of this excisable and eventually "transferable" population is explained by high cost that would be invested in the transfer event. Major strategies to assess the

stability and maintenance of certain GIs include limited replication, deployment of active partitioning systems, and the active killing of donor free cells due to either an abortive toxin-antitoxin (TA) infection system or a novel mechanism observed only in the SXT/391 family, the so-called "trapdoor." Recombination between elements occurs if they do not belong to similar exclusion clusters.

Bacteriophages and phage-related particles. Transduction events occur in nature up to an estimated 20×10^{15} times per second (379, 380). The role of bacteriophages and phage-related particles as reservoirs and drivers of AMR in the human and animal gut, sewage, and agricultural soils has been extensively studied (380–382). Mobilization of chromosomal AMR genes by transduction has been demonstrated for major opportunistic pathogens such as *Enterobacterales* (*E. coli* and *Salmonella*), although much more frequently in streptococci and staphylococci. In the latter cases, antibiotics at subclinical concentrations have been shown to promote the bacteriophage transduction of ARGs. However, network analysis revealed that plasmids connect bacterial cells more frequently than phages (383); the estimated transduction/conjugation rate is approximately 1/1,000 (380, 384).

Chromosomal ARGs are infrequently located in core genome regions, which are the common sites of prophage integration. Moreover, the cost of carrying ARGs might restrict phage evolution. When CRISPR-Cas immunity toward foreign DNA is borne by lytic phages, the host bacteria are prevented from acquiring plasmids, eventually carrying resistance determinants. Evasion of CRISPR immunity by plasmids occurs at the host level through high-frequency loss of functional CRISPR-Cas immunity, a frequency as high as 10^{-4} in the case of the conjugative plasmid pG0400, which encodes mupirocin resistance. However, CRISPR can be reacquired by HGT in environments where phages are a major cause of mortality (385).

Phages can combine with other MGEs, such as plasmids, transposons, and genetic islands, forming phage-like elements; phage-plasmids occur in ~7% of plasmids and ~5% of phages (386). One class of phage-like elements, called gene transfer agents, is based on the presence of usable capsids in the bacterial chromosome, facilitating mobilization of bacterial DNA (386), which can transfer AMR in heterologous recipients at 10^6-fold-higher frequencies than previous estimates of their transformation and transduction rates in natural environments. The host range, however, appears to be very concentrated in alphaproteobacteria from ocean environments, reducing the risks for human health.

Flow of Mobile Genetic Elements and Antimicrobial Resistance Genes

Most AMR genes are "mobile" because of MGEs. The term mobile here indicates the ability to be transmitted among heterogeneous bacterial entities. But to play a significant role in ecology and evolution, mobility should provide adaptive advantages for both ARGs and bacteria and also for the microbial community acquiring the AMR genetic trait. Mobility of AMR highly depends on the robustness (ability to tolerate irregular changes) and conduciveness (efficacy in reaching the goal of resistance) of the players facing different ecogenetic contexts. High robustness-conduciveness creates "highways" where AMR genes are maintained and circulate in a consistent, sometimes permanent manner. These facilitated processes are frequently derived from the historical biological background, balanced in multihost bacterial communities that provide the ecological continuity required for genetic interactions.

Environmental context of antimicrobial resistance genes flow. The resistance and resilience of a functioning ecosystem to the heterogeneous and often stochastic environmental fluctuations depend on the species' richness. The more diversity in the community, in the species and in the MGEs, the more chances to respond to irregular and sudden perturbations, increasing the AMR evolvability (387). However, the number of variants required to generate robustness can vary during evolution due to the low or infrequent temporal occurrence of the changes. Thus, the balance between robustness and evolvability drives the evolution of AMR entities (388). A major source of environmental variation derives from anthropogenic activities, which are increasingly considered in the analysis of AMR under the One Health, Global Health, and Planetary Health perspectives (389, 390).

Cell-free DNA as a source of ARGs has increasingly been reported at the interface of the human and water environments (391). Depending on the bacterial species involved and the gene transfer mechanisms that are active, a number of processes limit (or enhance) the uptake, the transfer, and the stabilization of foreign DNA in bacteria from different environments. The canonical HGT mechanisms of conjugation, transduction, and transformation involve genetically and ecologically connected populations (391, 392). Other HGT mechanisms are increasingly being documented in soils and marine habitats, such as DNA-packing extracellular vesicles and DNA transfer through intercellular nanotubes (393–395). The combination of various HGT processes is now recognized as a primary strategy for transmission and cooperation between natural bacterial communities in order to exploit genetic common goods, such as ARGs (284).

Highways for ARG flow vary according to the environmental factors, which have dramatically changed during the 20th century due to massive anthropogenic interventions. The release of antibiotics, heavy metals, pharmaceuticals, microplastics, and manure into the soil and water ecosystems is expected to greatly affect the composition and dynamics of resistomes and HGT events in nature. Major molecular effects from these stressors include triggering the SOS response, increasing reactive oxygen species levels, weakening the cell wall, modulating quorum-sensing processes, increasing adaptive AMR, and enhancing HGT (396, 397). The transient bacterial communities composing manure soils imply that transformation or phage transduction (also present in these environments) could have a relevant role (398). Kotnik and Weaver (399) have estimated that under contemporary ecological conditions, at least 10^{24} microorganisms are subjected to a freeze-and-thaw cycle, at least 10^{19} are subjected to sand agitation, and at least 10^{17} are subjected to conditions suitable for electrotransformation in any given year. Common minerals employed in animal food supplements and biosolids promote the direct transfer of AMR plasmids between bacterial species (399, 400). Most species involved in AMR are generalist and are thereby able to cross different higher host species (401, 402). The conduciveness of ARGs depends on MGE promiscuity, which is determined by either ecological opportunity (plasmids and other conjugative elements) or phylogenetic distance (bacteriophages). Each element employs preferential transfer mechanisms in which recipients and donors play different roles, determining preferential roads for antibiotic dissemination. Changes in reservoir size and in regions of transitions between environments (ecotones) can facilitate the emergence and persistence of pathogens and the AMR traits they carry, as has been reported for methicillin-resistant *S. aureus* (MRSA) and enterococci (403, 404).

Gene flow also occurs by natural transformation. Recipients (competent cells) play a central role in natural transformation. Naturally (heritable) occurring bacterial subpopulations with enhanced competence or recombination potential (mutator strains) have been associated with ARGs and MGEs in the various species frequently involved in AMR (405). Variability in competence is often explained by the phenomenon of phenotypic bifurcation or "bistability," stochastic events triggered by environmental stimuli, but now appears to be determined by highly regulated processes within individual cells (406). Environmental distribution and dynamics of competence phenotypes are still unknown.

The recombination of homologous or heterologous acquired DNA has been extensively discussed elsewhere (392). The contribution of DNA uptake in natural environments appears to have been greatly underestimated. The acquisition of transposons, integrons, and gene cassettes by competent disparate species (407) and the possibility of acquiring large fragments and antimicrobial-resistant genes (408) frequently occur. Recent studies that relate competence for killing nearby cells via fratricide or sobrinicide (in *Streptococcus*) or by kin-discriminated neighborhood predation (through T6SS systems in *Vibrio* and *Acinetobacter*) have revealed an active HGT strategy for acquiring exogenous DNA that can contribute to the fitness of the predator after acquiring beneficial adaptive traits, including the uptake of AMR plasmids (409, 410). Lastly, transformation has recently been suggested as a relevant process to rescue bacterial cells from selfish mobile elements (411).

The acceptability to foreign genes: barriers to gene flow. Donors play a central role in conjugation, whereas recipients often limit the transfer or the establishment of the conjugative elements. Transference is highly regulated in plasmids and differs between Gram-positive and Gram-negative species (412). Despite the differences in backbone, regulatory networks, and evolutionary origins, ICEs appear to have a relatively restricted host range and share a general model of bistability that explains their horizontal or vertical transmission (378, 412). Conjugative elements frequently interact with other elements within the cell and can modify HGT ability in recipients (411, 413). MGE promiscuity is related to this affinity requirement and to the availability of attachment sites in the recipient. Hot spots for a specific insertion site are common for biologically relevant GIs, transposons, and bacteriophages in species of *Actinobacteria*, *Firmicutes*, and *Proteobacteria* (e.g., the 3′ end of the housekeeping gene encoding glutamine aminotransferase [GMP synthetase]), although such specificity can be relaxed, facilitating uptake at secondary sites (414).

Depending on the bacterial species and MGEs involved and the gene transfer mechanisms, a number of processes limit (or enhance) the transfer, uptake, and stabilization of foreign DNA molecules in bacteria. Recipients already carrying conjugative elements limit the acquisition of similar entities by incompatibility (plasmids) and exclusion (plasmids and ICEs). Plasmid incompatibility is often modified by recombination, which explains the frequent coexistence of similar plasmids in antibiotic-resistant bacteria, such as F plasmids in *E. coli* and pheromone-responsive plasmids in *E. faecalis* (319, 415, 416). Incompatibility also affects the dynamics of ICEs and plasmids with the same replication machinery (417). Surface/entry exclusion affects plasmids and ICEs of differing GC contents (418). Whereas surface exclusion prevents close contact between cells, entry exclusion prevents DNA transfer after the formation of the mating pair.

Defense systems prevent the introduction of heterologous DNA from conjugative elements and phages and are classified into two major groups, namely, immunity and dormancy induction-programmed cell death, which can be collected, analyzed, and visualized in a comprehensive prokaryotic antiviral defense system database comprising elements from more than 30,000 species (https://bigd.big.ac.cn/padsarsenal). The immunity group includes RM systems, bacteriophage exclusion systems, and clustered, regularly interspaced, short palindromic repeats adjacent to *cas* gene (CRISPR-Cas) systems. The dormancy induction or programmed cell death includes TA systems and abortive infection (419, 420). Defense mechanisms show nonrandom clustering suggestive of nonadaptive evolution of the islands through a preferential attachment-like mechanism underpinned by addictive properties (421), which can eventually act as selfish mobile elements.

Barriers between different prokaryotic groups influence antibiotic resistance gene flow. Phylogenomic networks employing genomes and metagenomes reflect the major impact of HGT during microbial genome evolution, suggesting barriers at multiple levels between various prokaryotic groups (422–424). Phylogeny correlates with ecology, the field of ecophylogenetics, and phylogenetic community ecology. If ARGs are expected to exist virtually everywhere, they are selected and circulate and evolve preferentially among phylogenetically related organisms not only because of their ecological coincidence but also because they have been evolutionarily adapted to the genetic background and physiology of groups sharing a common ancestor. Genes recently acquired via HGT are more similar in codon usage than genes that have been vertically inherited (425); for instance, recently acquired genes tend to be relatively AT rich compared with the host's chromosome. The phylogeny of RM systems also correlates with the phylogeny of the bacterial taxa; these mechanisms against foreign DNA create preferential pathways of genetic exchange, within and between lineages, with related RM systems (426). Transferred genes are concentrated in only approximately 1% of the chromosomal regions (126), and the density of chromosomal hot spots for integration of foreign genes in different species should therefore influence the acquisition of ARGs.

However, HGT occurs at a lower frequency across diverse bacterial phyla (427) linking distinct genetic pools (384, 428, 429). Barriers to HGT among distantly related

bacterial species are still poorly understood but are thought to depend on the transfer mechanism (broad-host-range MGEs) and community permissiveness, which refers to a community's ability to share a gene acquired by HGT (genetic exchange communities). Ecologically cohesive bacterial populations forming a multispecies community should have better chances to establish a "common good," ensuring the resilience of the community partners involved in cooperative functions (430). The analysis of networks focused on genes shared between chromosomes of different species, plasmids, and phages shows not only that genes are preferentially shared between groups of closely related genomes and between typologically consistent groups, as in phages with phages and plasmids with plasmids, but also that most gene transfers occur within particular geolocalized habitats (31). As can be expected, ecologically isolated populations (including many intracellular bacteria and those tolerating unique stressful environments), which are also in genetic isolation, are less prone to receiving ARGs (431). Cooperative or competitive-amensalistic interactions between species should influence co-occurrence at short distances and HGT.

Interactions between coexisting MGEs are common and might constitute barriers for gene flow. Most bacterial pathogens host a multiplicity of potentially interacting MGEs (311), obtained by sequential or simultaneous acquisition or by long-term local plasmid evolution. These interactions can alter, among other things, MGE transferability and maintenance. Mobilizable plasmids, which comprise at least 25% of all plasmids, rely on other conjugative elements present on the host cell to be able to spread by conjugation (432, 433). Conjugative plasmids might also facilitate the conjugation of another conjugative plasmid present in the cell, a phenomenon that frequently involves plasmid-plasmid RecA-dependent cointegration, sometimes using common transposable elements (434). However, facilitation of the transfer of a coresident conjugative plasmid does not necessarily involve conventional RecA-dependent recombination. Facilitation is negatively influenced by the surface/entry exclusion but enhanced by favoring donor-receptor "mating clumps" mediated by plasmid-encoded sex pili (435). MGEs affect the fitness effects produced by other MGEs coexisting in the same cell. Plasmids, for example, typically engender a fitness cost in the host bacterium (304, 436); however, these costs can be ameliorated (positive epistasis) or accentuated (negative epistasis) by the presence of additional MGEs (311, 437). Epistatic interactions between MGEs can determine the fate of the MGE in bacterial populations, promoting low-fitness-cost associations and long-term maintenance, thus shaping the highways of AMR genes (311, 438, 439). Plasmid evolutionary success and the plasmid-mediated spread of AMR are to a significant degree the results of intracellular plasmid competition with other plasmids, influencing spread by lateral transfer, in particular, stable plasmid inheritance (incompatibility) (440). Conjugative plasmids commonly encode fertility inhibition determinants, which reduce the conjugation frequency of other plasmids present in the same cell (441). Plasmid incompatibility is based on common regulatory mechanisms of coexisting plasmid replication, resulting in a competitive replicative dynamic leading to the loss of one of the plasmids in the cell progeny. Replicon typing has served as a method for classifying plasmids (*Inc* or *Rep* typing) (442). However, there are numerous examples in natural bacterial isolates of incompatible low-copy-number conjugative plasmids carried jointly, providing evidence that resistance plasmids can solve incompatibility, increasing the cellular repertoire of ARGs (435). Incompatible plasmid coexistence can result from cointegration or from plasmids harboring more than one mode of replication (321). Plasmid localization and partition (Par) systems also cause plasmid incompatibility, such that distinct plasmids with the same Par system cannot be stably maintained in the same cell (443). In addition, TA systems can eliminate incompatible plasmids from the progeny (444).

A fascinating example of how interactions between MGEs affect their horizontal transmission is the arms race between phages and pathogenicity islands in *Staphylococcus aureus*, in which both elements compete using a complex repertoire of molecular interactions packaged in the phage capsid (445). Other examples of

interactions among MGEs occur among pipolins, self-synthesizing transposons encoding replicative B type DNA polymerases, which can be present in *E. coli* but are not involved in AMR, and other integrons (446).

Species are expected to create natural barriers for gene flow. However, the concept of species remains elusive in bacteriology (447–449). In the age of whole-genome sequencing, it is widely accepted that strains belong to the same species if they share more than 95% average nucleotide identity. Although MGEs belong to the accessory genome and considering that there is a common evolutionary history for MGEs and their usual hosts, a mutual adaptation has taken place. However, many plasmids (more than 50% of those examined by bioinformatic methods) can colonize species from different phyla (302). In any case, it remains true that the same type of MGE tends to be associated with the same type of host (450). Historical coexistence with MGEs has likely contributed to speciation (or at least with the gene regulatory mechanisms that impose "styles of life") in a particular ancient host. Plasmid stabilization is likely to occur in particular bacterial hosts, mediated by different mechanisms, such as mutations in a replication protein gene, acquisition by the resistance plasmid of a transposon from a coresiding plasmid encoding a putative TA system, and a previous mutation in the host's global transcriptional regulation genes (451). The process of stabilization by mutation of the plasmid replication protein involves the emergence of numerous plasmid variants differing in this initiation protein; clonal interference (competition between variant clones) thereby determines the evolution of the persistence of drug resistance (452). Plasmid-encoded TA systems have an advantage in within-host plasmid competition if the host cell is sensitive to the toxin (453). Long-term coevolution of a plasmid in a particular species can result in partially or fully codependent replicons, a "plasmid specialization in particular species" limiting the spread to other lineages in which the maintenance or expression of plasmid traits, such as ARGs, could be reduced (307, 454). Most bacterial species tend to diverge into subspecies and clones by the process of "clonalization" (mimicking speciation by niche adaptation where HGT is involved) to neighboring ecological niches (ecovars and ecotypes) (449). This ecological neighborhood facilitates the evolution of plasmid host specificity, frequently overcoming the process of clonalization (455).

Intracellular genomic dynamics of MGEs. MGEs such as ISs and transposons can move almost randomly (sometimes with associated ARGs) from one location to another within the genome (chromosomes or plasmids) of a bacterial cell. Integrons employ site-specific recombination to transfer resistance genes between defined genomic spots. Averages of 10^{-4} IS insertions and 10^{-5} IS-mediated recombinations per genome have been estimated per generation in the *E. coli* K-12 genome (456). How are ISs maintained successfully in bacterial organisms despite transposition bursts frequently being deleterious to their host genomes, often induced by stress, including antibiotic exposure? The intake of ISs through the uptake of MGEs is insufficient to replace lost ISs; however, continuous adaptive genetic variation resulting from insertion events can be maintained as "evolutionary insurance" for bacterial adaptation to changing environments, which could facilitate homologous recombination, removal of deleterious genes, and acquisition of advantageous mutational events (351), as well as ensuring cross talk between genetic regions of the cell, sometimes from different intracellular replicons. As previously shown, ISs (and composite transposons) are associated with the acquisition of ARGs (288).

Integrons are extremely ancient and widespread groups of chromosomal elements with low basic diversity (only three main classes associated with broad bacterial taxa but with many variants). Integrons are not MGEs in their own right, given that the integron integrase cannot excise its own gene from a chromosome; however, integrons can gain mobility (mobile integrons) through intracellular association with transposons or plasmids and can carry ARGs (457). Integrons also act to efficiently capture exogenous genes ("adaptive on demand" genes, including ARGs) that are acquired (and excised) as "gene cassettes," expressed under the function of an external promoter. It

is unclear how genes that originate in different species and environments reach and are recruited by the integron; however, the acquisition of mobile integrons carried by plasmids or mobile transposable elements could play a relevant role (458). The order of gene cassettes in the string (possibly hundreds) can be changed, thereby altering the distance to the promoter (289). Mobile promoters can be horizontally transferred (458) and can sometimes influence the expression of ARGs by intragenomic mobility (459). MGE dynamics is regulated by the cell to reduce "intragenomic conflicts," ensuring a maximum tolerated number of copies, from plasmids to transposable elements, as occurs in transposon immunity (460).

Intracellular interactions between plasmids and the chromosome also constitute a relevant topic. Hypothetically, the translocation of advantageous genes from the plasmid to the chromosome, followed by a "costly" plasmid loss in the progeny could keep original fitness (461); however, as plasmid loss is frequently minimized by compensatory evolution, this reduces the ARG chromosomal gene capture (462). Small plasmids with a high number of copies per cell should be more difficult to eliminate than large plasmids with a small copy number. A debatable issue is whether small plasmids impose a different fitness cost than large ones; however, meta-analysis studies have suggested that there is not much difference. The fitness cost is proportional to the number of ARGs carried in the plasmid, suggesting that plasmid loss should be more frequent in multiresistant plasmids (439).

We have highlighted the multiple, almost unlimited wealth of intracellular interactions among MGEs and with the bacterial chromosome, which creates a scenario of overwhelming complexity, in which a multiplicity of genetic combinations is constantly created and offered to natural selection in various environments. These experimental combinations can surpass the normal mutation rate and can also impose a lower fitness cost for the cell in the medium and long terms. Plasmid carriage has a lower average fitness cost than chromosomal mutations (439). The fuzzy ontology of MGEs, where the interaction among phages, plasmids, and transposons produces a "mosaic continuum," provides an accurate image of this "intracellular evolution laboratory" (463). A good example is the unpredictable structure of mosaic plasmids (50% of all represented in databases), composed of genetic elements from distinct sources and unevenly distributed across bacterial taxa, although possibly more common in more environmentally connected species (464). The genetic diversity of mosaic plasmids has contributed to the selection and spread of AMR (415, 465) but has increased entropy while considering evolutionary trajectories.

ECOGENETICS OF ANTIBIOTIC RESISTANCE ACCESSORY GENOME

MGEs should be transferable from a donor to a receptor bacterial host, a transfer that depends on the autonomous ability of the MGE to encode its own transfer mechanisms or to be mobilized in *trans* by another MGE. Most transmissible ARGs should correspond to the "accessory genome," and the trajectories of genes belonging to the mobile accessory-adaptive genome should correspond to ARGs, which is illustrated in Fig. 7 and detailed below.

Accessory Gene Trajectories in *Gammaproteobacteria*

The gene flow trajectories in *Gammaproteobacteria* are clearly related to the species' phylogenetic neighborhood. Accessory gene flow analysis among *Gammaproteobacteria* reveals (Fig. 7) a "core ensemble of species" in *Enterobacterales*, constituted by *Escherichia*, *Klebsiella*, *Salmonella*, *Citrobacter*, and *Enterobacter*, followed in descending order by *Serratia* and *Yersinia*, *Pasteurella*, *Haemophilus*, *Vibrio*, *Acinetobacter*, *Pseudomonas*, and *Legionella* (200). These accessory gene exchange ensembles correspond closely to phylogenetic groups. In principle, accessory (and resistance) gene spread should be facilitated among members of the same phylogenetic ensemble, such as the *Escherichia-Enterobacter* clade, composed by *Escherichia*, *Klebsiella*, *Enterobacter*, *Raoultella*, *Kluyvera*, *Citrobacter*, *Salmonella*, *Leclercia*, and *Cronobacter*. Other *Enterobacterales* clades include *Erwinia-Pantoea*, *Pectobacterium-Dickeya*, *Serratia-Yersinia*, *Hafnia-Edwardsiella*, Proteus-*Xenorhabdus*, and *Budvicia*. Ecological distancing

affects bacterial interactions, and an eco-phylogenetic approach might be established to predict significant gene flow.

Accessory Gene Trajectories in *Firmicutes*

In *Firmicutes*, the accessory genome clusters are more dispersed than in *Gammaproteobacteria*. Stronger interactions are found among a core of *Streptococcus*, *Enterococcus*, and *Staphylococcus* clusters and weaker interactions are found with *Clostridioides*, *Bacillus*, *Clostridium*, *Lactobacillus*, and *Leuconostoc* clusters. However, all these clusters share accessory genes and, potentially, ARGs. The structure of these interactions fits well with the protein content network of AMR proteins found in the plasmids and chromosomes of *Firmicutes* (466). As in the case of *Gammaproteobacteria*, gene flow is highly dependent on the ecogenetics of the various species, e.g., *Listeria*, which, despite being in the vicinity of the *Streptococcus-Enterococcus-Staphylococcus* exchange cluster, undergoes infrequent acquisition of accessory genes and resistance genes from phylogenetically related species (431).

Phylogenetic Context of Gene Flow: Coselection of Kin Populations

Horizontal gene transfer might be favored by phylogenetic proximity, but at the same time, HGT contributes to shape genetic unrelatedness, i.e., diversification. For instance, when plotting presence/absence of large genetic elements onto extensive phylogenetic trees, it becomes evident that some elements are present in all isolates of a given phylogenetic sublineage. This implies that acquisition of the large element was part of the shaping event for the fitness of that clone. This observation is coupled to the fact that the same mobile element may not be present in any other major clone (467). A possible explanation is that the lateral gene acquisition provokes asymmetric fitness among the potential recipients; we cannot exclude that HGT events are extremely frequent but aborted (reach an evolutionary dead end) in most cases.

How does relatedness between bacterial lineages influence linked evolutionary processes? In a certain sense, the evolutionary success of a member of a given lineage group is the success of this group in competition with other groups. The winner, typically the best-adapted clone, was probably positioned by previous successes of the group in the circumstances that facilitated its own selective advantage, a feature that can be considered a "group investment" in the success of one of its members. This investment should now produce a return for the benefit of the winning kin-related members of the group. Ultimately, the evolutionary advantage frequently benefits the entire group. How is the evolutionary benefit redistributed? If the winner protects the whole group by producing molecules protecting from antibiotics the bacterial ensemble, or offers transformable sequences with target site alteration, or MGEs, then HGT plays a major role here. The winner increases in population size and redistributes the acquired trait among the kin members (relatives) of its group.

Recombination, the biological process of exchange of DNA sequences, has profound effects on the evolution of bacterial genomes and AMR. Recombination creates chimeric genomic sequences and can unite beneficial genes (or mutations) that emerged separately (468) or facilitate spreading of ARGs across bacterial populations (469). However, not all genomic sequences are equally likely to recombine. Recombination requires similarity between the DNA sequences to be exchanged (range of 20 to 100 nucleotides) (470, 471) and thus occurs more frequently between gene families sharing significant homology. Recombination of ARGs creates new allelic variants or mosaic genes in which mutations with different evolutionary origins merge. Examples include the widespread mosaic genes encoding resistance to tetracycline, aminoglycosides [*aac*(6′)/*aph*(2″), *aac*(6′)-Ie-*aph*(2″)-Ia, and *ant*(3″)-Ii/*aac*(6′)-Iid], β-lactams ($bla_{\text{LRA-13}}$, which is a fusion of a class C [AmpC-type] and a class D [OXA-type] β-lactamase; bla_{TEM} and bla_{SHV} β-lactamases), quinolones (*qnr* genes), or trimethoprim (*dfr* genes). These fusion proteins expand the substrate range beyond that of either domain alone, highlighting the important role of recombination in the evolution of AMR.

The adaptive success of gene transfer depends on the compatibility (relatedness) of the incoming gene (function) and the existing network of functional interactions in the recipient

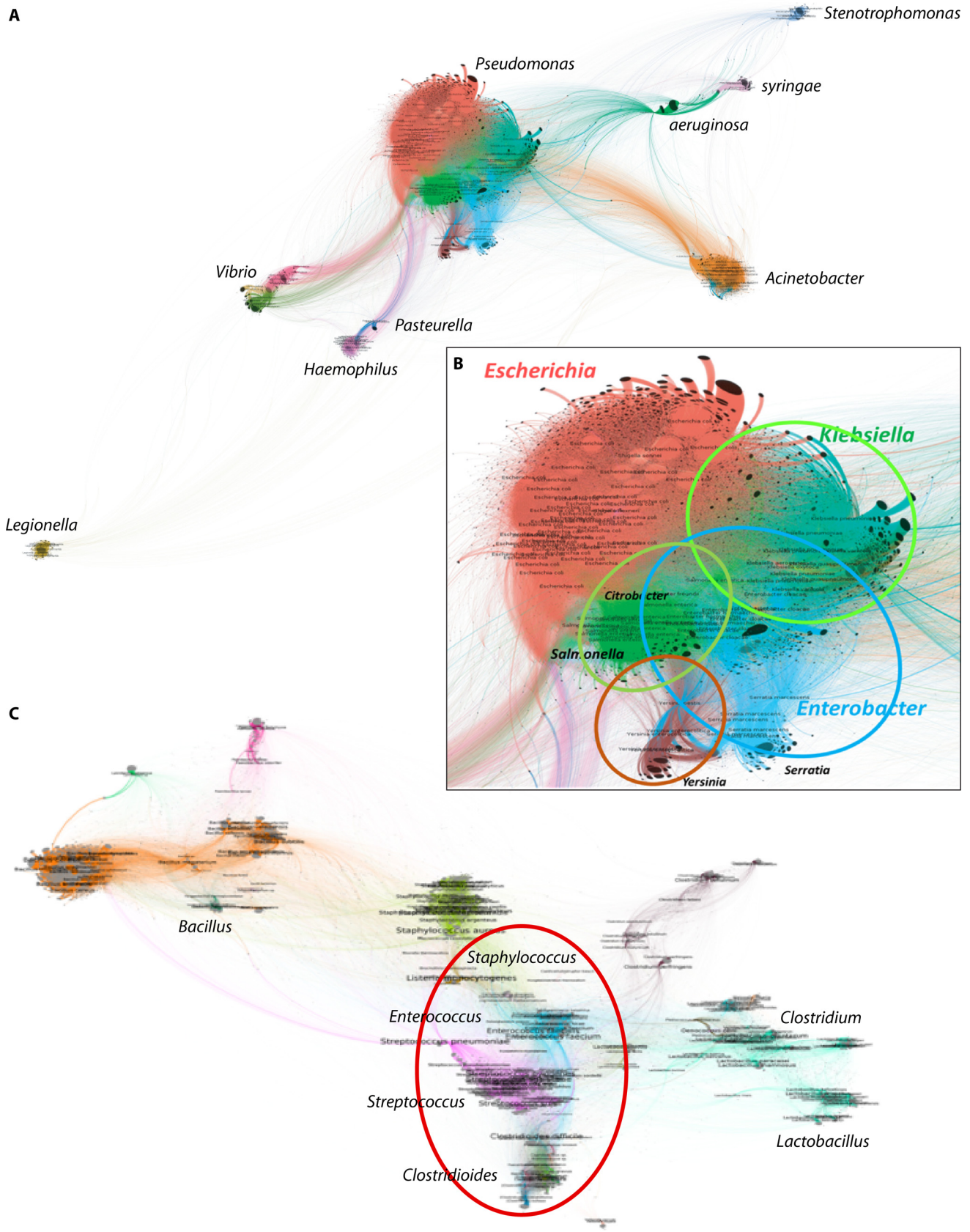


FIG 7 The flow of resistance genes among bacterial species should correspond to the flow of accessory genes. Shown are bipartite networks illustrating the accessory gene (protein) flow among species (genera) of the major taxa of *Gammaproteobacteria* (A and B) and among *Firmicutes* (C). Connections

(Continued on next page)

cell, as in physiologically coupled genes (472, 473). Nevertheless, the opposite can also occur if the product of the new incoming gene competes with a functionally relevant orthologous gene present in the genome of the new host and if the fitness costs are high because of this competition. In this case, gene decontextualization and exaptation can impose a lower fitness cost, allowing the acquisition of resistance genes from nonrelatives. In general, successful gene transfer is more likely to occur between organisms of similar GC contents (less than 5% difference for 86% connected pairs) (474) and/or involving plasmids able to bridge close to distant chromosomal backgrounds (384, 475).

A heterogeneity of phenotypes is expected to occur in time in a sufficiently large bacterial population derived from a single-lineage population, giving rise to a multiplicity of subpopulations that maintains high relatedness but not full identity. The important question here is whether these subpopulations will compete among them or, on the contrary, whether the members of this community of closely related strains will cooperate to gain common ecological advantages. It has been shown that significant signal interactions (including specific transcriptomic modulation) can occur between closely related strains (476).

The "gain" for the "group of kin populations" expresses the evolutionary weight of indirect selection. Those organisms directly selected, e.g., because they are resistant to antibiotics, promote the indirect selection of kin, genetically related populations, according to the classic statements by Fisher, Maynard Smith, and Hamilton (477). The "Hamilton rule" indicates that the fitness of the group of kin populations is the sum of those that have been directly and indirectly selected, and the benefit of those indirectly selected is proportional to the relatedness with those directly selected. Interestingly, the altruist population (the one that has been directly selected due to its AMR) and the cheater populations taking advantage from the altruist might reverse roles over time, when one of the cheaters might be directly selected and converted to an altruist and could then indirectly select the old altruist. This is a key concept for the "community selection" (as in the case of a species and their clones), based on bet-hedging adaptive strategies.

EVOLUTIONARY TRAJECTORIES OF RESISTANT CLONES AND SPECIES

ARG evolutionary pathways and trajectories and their involved MGEs are inserted into the evolutionary events of the bacterial clones, species, and communities harboring these genes. For the purposes of studying AMR, clones are subspecific discrete (distinct) lineages of highly related strains, called clonal complexes (CCs) (478–480). These clonal complexes conceptually resemble ecotypes that can be defined as sets of strains using approximately the same adaptive space, so that a novel or emergent genotype (mutant or recombinant) outcompetes other strains within such an ecotype (481). A limited number of specialized lineages within bacterial species have been amplified under antibiotic selection and greatly contribute to the worldwide spread and transmission of antimicrobial resistance. These lineages are known as "pandemic clones" and "high-risk clonal complexes" (HiRCCs) (482, 483) among public health and clinical microbiologists, respectively. In fact, they are fluctuating ensembles of kin clones with periodic emergences of new genotypes.

Evolutionary Dynamics of Resistant Clones

The contribution of population structures and environmental changes to the maintenance of genetic diversity in bacteria has been highly debated in the framework of two major dynamic models: the Red Queen hypothesis (RQH) and the stationary model (484, 485), respectively. The RQH states that populations are structured by biotic

FIG 7 Legend (Continued)
between two bacterial species indicate that the same accessory gene is shared, and the distance between the species (genera, in italics) is proportional to the number of connections. (B) Detail of the "core" of *Enterobacteriaceae* species sharing accessory genes; trumpet-like patterns on the surface of some clusters correspond to accessory genes that are unique for a particular strain (not connected with any other). The colored circles in panel B indicate the blurred borders of the species more frequently sharing accessory (and resistance) genes in *Gammaproteobacteria* and the "core" group exchanging accessory (and resistance) genes in *Firmicutes*.

interactions, in such a way that one population (or genotype) changes the environment, forcing the others to continue evolving "to keep the place" where they were originally adapted. Initially, the RQH implies a constant rate of evolution based on successions of single populations with a common ancestor, as in the classic periodic-selection model. Classic periodic selection purges diversity by the emergence of adaptive genotypes as sequence types (STs) that outcompete the ancestor strains, but the concept applies to clonal complexes and ecotypes (486). Other recent more inclusive models allow progressing in time through coevolutionary oscillations involving several coexisting populations (487–489). In the stationary model, changes in the population structure are "punctuated" and occur abruptly in response to environmental disruptions after relatively long periods of stasis (485, 490). The RQH and stationary models are, respectively, allied to the "gradualism" and "punctuated" end views of evolution. However, these major evolutionary models are not mutually exclusive, and periods of accelerated evolution coinciding with environmental disruptions can occur. In all these models, the "ancestor" trunk coexists with the diversified branches, which is suggested by phylogenetic studies (73). A "killing-the-ancestor" kinetics by more recent lineages, thereby accelerating evolution, cannot therefore be ruled out (491). The population structure is also dependent on another important diversifying force, negative frequency-dependent selection, which probably contributes together with subniche adaptation and niche construction (449, 492–494). Finally, when the environment of a local ensemble of bacterial populations is dominated by a hierarchically superior biological entity, and such interaction has been stabilized by protocooperation or symbiosis (e.g., microbiota inside a human or animal host), the evolutionary dynamics of clones and species is also regulated by the homeostasis of the host, maintaining microbiota on a leash, eventually leading to coevolutionary and coregulatory processes and ensuring the functional resilience of the interaction.

Hence, the evolutionary trajectories of antibiotic-resistant organisms are inserted into other evolutionary trajectories; for instance, the evolution of AMR organisms inhabiting mammals follows the evolution of the mammals themselves. In a single species (such as humans), the evolutionary trajectories of a particular antibiotic-resistant lineage are determined by the changing ecology of the individual and local group microbiota, the result of conditions such as aging, feeding habits, health status, local environment, hospitalization, drug exposure, and, most importantly, exposure to antimicrobial agents. Extinctions are rare, and vulnerable genotypes can persist as residual populations, survive different periodic-selection rounds, and be "rescued" and further amplified (495).

An unresolved issue is whether species diversification is the result of specialization in highly specific niches, which might finally limit their spread, implying a reduction in population sizes and a higher risk of extinction. There is the possibility of rapidly inverting this risky evolutionary trend by exploiting neighboring niches and compensating specialization with complexity (81, 496), or because of environmental changes. Such rapid adaptation to avoid extinction is known as evolutionary rescue, a term coined in 1995 with roots in the works by Haldane and Simpson on the evolution time frame (497, 498). According to theory, the likelihood of clonal populations being rescued depends upon the population size, the supply of genetic variation, and the degree of susceptibility to stressors (499). The rescue process is influenced by epistasis, HGT (500), recombination (501), the cumulative history of stress, general environmental changes, and the population structure, which includes clonal interference (502).

However, evolutionary rescue under antibiotic exposure likely occurs because minority resistant cells, releasing antibiotic-inactivating substances, protect the other neighbor (susceptible) cells, which are spared the biological cost of producing the resistance trait, which reduces the amount of antibiotic in the environment where the susceptible bacteria are placed and is therefore converted into a "public good." For instance, a minority of beta-lactamase-producing *E. coli* cells inside a colony are able to protect the whole population, including a majority of antibiotic-susceptible cells, from

a beta-lactam antibiotic (503, 504). Such indirect resistance occurs for most antibiotic-modifying or -degrading enzymes, including those acting on macrolides, tetracyclines, and chloramphenicol. These types of collective relations have been examined on the basis of game theory (505). In principle, the minority that produces the "common good" resistance should be at a disadvantage, given that it concentrates all the costs; the other cells are "cheaters" which have benefits but no costs. This relationship is, however, dependent on the antibiotic concentration, because the resistance mechanism protects the producers more than the neighbor cells. However, the important evolutionary fact is that the resistance trait is frequently located in a transmissible genetic element. By maintaining life in the plasmid-free part of the population, these cells might act as recipients of the beta-lactamase-encoding plasmid, so that the proportion of cheaters will progressively decrease (even more so if the antibiotic concentration rises). At some point, many cells will be producers, and the common good (in large amounts) will then favor the survival of neighboring susceptible bacterial populations. The release of "common goods" favors the survival of the entire population, even if there are no cheaters within. For instance, many antibiotics show an "inoculum effect" such that a dense population tolerates much higher antibiotic concentrations (higher MICs) than diluted or isolated cells (506). Thus, the best way to observe the intrinsic activity of drugs is to expose single cells to various antibiotic concentrations to obtain single-cell MICs, an approach that might help detect the first steps of mutational resistance selection (507).

Structure of Clonal Fluctuations

A common observation in studies on the epidemiology of AMR is the frequent shifts in the prevalence of bacterial clones, giving the appearance of "oscillatory replacements" or "clonal waves." The reasons involved in these changing dynamics, the "structure of the variation," frequently remain obscure. As an approach to this topic and inspired by the classic concept of periodic selection, Fred Cohan defined types of molecular adaptive changes that determine the frequency of ecological diversity within and between populations (495).

Between-host and within-host diversification. Diversity can be fostered by host invasion, given that variation can increase by adaptations to new hosts. How might a "foreign" invader outcompete (or a least coexist with) well-adapted local strains? One possibility is through genetic variation finding an unexploited niche in the new host that was disregarded due to the success of commensal strains. If fitness is low at the start, the strain can increase in abundance, following something akin to the Sewall Wright metaphor of the shifting-balance theory (applied to species) (508). The possibility of resistant clones crossing barriers between hosts has major consequences on the evolution of AMR. Transmission between hosts implies bottlenecks; i.e., frequently only a sample of the clonal composition is transmitted, which favors the spread of particular clones, either stochastically (nonselective bottlenecks) or in a deterministic manner (selective bottlenecks), when the receptor host is suitable to be preferentially colonized by a particular clone (104).

There is a variability of niches among hosts and within hosts that drives the variability of antibiotic-resistant clones, which correspond to "Hutchinsonian niches," imaginary multidimensional spaces in which each dimension represents the variable range of a particular environmental condition or resource required for the optimal growth of a sublineage or particular genotypic group (449). Ecological niches are constructed by the hosts and by the bacterial organisms that live there, creating subniches and neoniches that can be exploited by new bacterial genotypic variants (449). Clonal/strain adaptation to new niches involves strategies of competition and cooperation with other microbes. Within the same lineage, a certain cooperation of adaptive processes, including mutation and recombination, can be expected (509). Thus, clonal interference is not absolute, allowing for the coexistence of several clones with beneficial (adaptive) mutations that might reach relevance with an increase in population size. However, if clonal interference is high, recombination might allow for the maintenance of more

beneficial changes in a lower number of clonal entities (502). In a reduced number of intraspecific clones but with higher cell densities, HGT might favor natural selection of adaptive traits, including AMR.

Variation fostering cloud or bunch clonal selection. The variation fostering cloud or bunch clonal selection can be considered the opposite of the case presented in the previous section. Adaptive genetic variation might confer an advantage favoring several populations, particularly for kin clones but also for species sharing the same or neighboring niches, resulting in "cloud" or "bunch" simultaneous selection of different bacterial ecotypes. HGT is frequently involved in this process; MGEs serve as vehicles for "common goods," in our case ARGs. Such "bunch" adaptations tend to purge the neutral sequence divergence both within and between populations while preserving the distinct DNA sequence similarity of a population/cluster. A poorly explored but interesting possibility is whether the selection of a particular clone leads to a "niche construction process" (449), which might facilitate the acquisition of coexisting kin-related clones, an effect that could have a strong influence on the epidemiology of AMR.

Clonal variation triggering community selection. Local clonal diversification is dependent on the local diversity of Hutchinsonian niches (see above), but such niche diversity is dependent, in turn, on the whole structure of the microbial ecosystem, such as microbiota. In a sense, globality is an ensemble of many localities, a concept that suggests the existence of a "selection of global communities" improving the resilience of the ensemble when confronted with external variation. Clonal evolutionary trajectories are also determined by the host-microbiota macroenvironment, subjected to external and internal processes, such as trophic interactions (the "intestinal chemosphere") and competitive interactions, leading to multilevel self-organization (510).

From clonal diversity to clonal fluctuations. How large is the clonal diversity within bacterial species that are able to harbor significant ARGs? In the case of *E. coli*, fewer than 10,000 STs have been identified in multilocus sequence typing (MLST) databases. However, in 1992, Orskov and Orskov estimated an *E. coli* diversity ranging between 50,000 and 100,000 serotypes (511). Taxonomy based on single-nucleotide polymorphisms can be too fine-grained a technique to discern clones. How many *E. coli* clones coexist in a single individual host? Current data suggest that an average of 3.5 genotypes are recovered per host, with some hosts having 6 genotypes (512, 513). These data probably underestimate the clonobiome diversity of *E. coli*. Determining a species' clonal diversity per individual and its evolution over time is not a trivial task, but determining these phylogenomic aspects is of relevance to understanding the evolution of AMR.

Clonal fluctuation in human populations can be better documented for well-adapted species belonging to the normal microbiota when linked to epidemic events. For instance, clonal shifts in *Salmonella* are highly influenced by events in food safety and food markets and agriculture; changes in the frequency of clones belonging to the major *E. coli* phylogenetic groups, from A and B1 in the 1980s to B2 and F in the 2000s (496, 514), or phylogenomic groups in *Enterococcus* populations (515) in Western countries, illustrate the phenomenon of bunch clonal selection previously mentioned. Clonal fluctuations may also associate with interventions such as the implementation of massive immunization programs with pneumococcal conjugate vaccines (PCV) that led to serotype replacement or serotype switching of *S. pneumoniae* AMR populations (516, 517). Clonal fluctuations should be differentiated by individual hosts (age ranges are a critical issue), groups of individuals (e.g., particularly the type of hospitalized patient and human communities in different social-environmental conditions), and larger entities (studies in a single hospital or numerous hospitals, regions, and countries).

Clonal fluctuations resemble wave kinetics and occur at the individual level (inside a single host), in groups, and in large host communities, forming landscapes of waves of different amplitudes. In the individual and particularly in open ecosystems (such as mucosal membranes), a bacterial species structure implies the coexistence of several clones, each one adapted to the situation of a particular spatial-temporal environment, ensuring species resilience: an "optimal clonal composition." Coexisting clones can be conceived of as alternative stages of the species' population. Due to the fluctuating

conditions, certain cells of the best-adapted clone at a given moment will multiply at high growth rates creating the expansive, leading edge of a pulling wave, resulting in increased cell density. The increased bulk of the wave probably contributes to pushing the wave forward, with the result of replacing other clones (Fig. 8). The "wave" study applied to the understanding of fluctuations in the spatial spread of biological invasions is a promising field of theoretical research (518, 519). In the case of AMR, the acquisition of resistance in the rising clone might provoke a collapse of other clones. A poorly explained problem in the dynamics of AMR is how the dominant resistant clonal waves of an individual host influence the invasion of other hosts in the group, producing confluences with similar waves and resulting in larger coupled waves that might increase fluctuations over large distances, as has been detected in other systems (518). The high geographical propagation velocity of certain high-risk clones suggests the possibility of this "potentiation by coupling waves" hypothesis (519). The HGT of adaptive genes (including AMR) from the first successes might "convert" other coexisting clones in cosuccessful resistant clones, as occurs in cryptic biological invasions (520). Spatial range expansion, the ability of a population or species to disperse and colonize novel areas, is a driver of variability, admixture, and rapid evolution, particularly during the initial stages, with changes in the evolution of cooperation (521–523). Traits favoring growth on expanding range edges tend to accumulate locally by this type of "spatial sorting," generating novel phenotypes (524). However, mixtures might also produce competition, provoking a persistent "mosaic of maladaptation" in which traits are not distributed in a pattern consistent with adaptation (525). In any case, if the parameter of "time" is the key dimension in evolution (526), a timeless-like biology is conceivable, based on the "flow of space" and the resulting consequences for living organisms (527). Clonal waves are pushed by the flow of time into a flow of spaces.

EVOLUTIONARY TRAJECTORIES OF ANTIBIOTIC-RESISTANT COMMUNITIES

Microbial communities (including microbiomes) are also evolutionary individuals when they are interactively associated with a particular environment and act as other entities of lower range in the biological hierarchy. As with other units of evolution, microbial communities evolve by trade-offs between dispersal and colonization, related to r-type and K-type strategies (528). We understand here as "antibiotic-resistant communities" those microbiotic ensembles that have been modified in composition because of antibiotic exposure and AMR. Short-term, transient modifications of these communities promoted by antibiotics are frequently reversible. If resistant organisms become prevalent, however, they might cause long-term or even permanent changes in microbial communities. In fact, this is one of the more severe global threats related to AMR, linked in this case not necessarily with human health but with the global health of the biosphere.

A modular organization of communities into subsystems constituted by groups of species contributes to their stability (529). Their modular ecological structure has probably evolved due to the cost of maintaining network interactions (530). Antibiotic challenge can result in a reestructuration that modifies species interactions, communities, and ecological dynamics. First, genetic variation affects communities; second, multispecies interactions cause diffuse selection and geographic mosaics of selection; third, there are macroevolutionary consequences of multispecies interactions (531). Most ARGs, including those acquired by HGT, occur in minority populations of microbiota, which can rise to "abnormal proportions" within their communities. As previously stated, this increase in relative population density frequently leads to clonal diversification, contributing to a more effective exploitation of the environment and an improved and more permanent adaptation of these clones to the environment (phylogenetic clustering). The net result is that these "better-adapted clones" emerge and can be maintained even in the absence of antibiotic exposure. The change in proportions of certain focal taxa (as the resistant ones) exert an "ecological pressure" on the rest of the community and should be "reshaped" in composition to ensure the maintenance

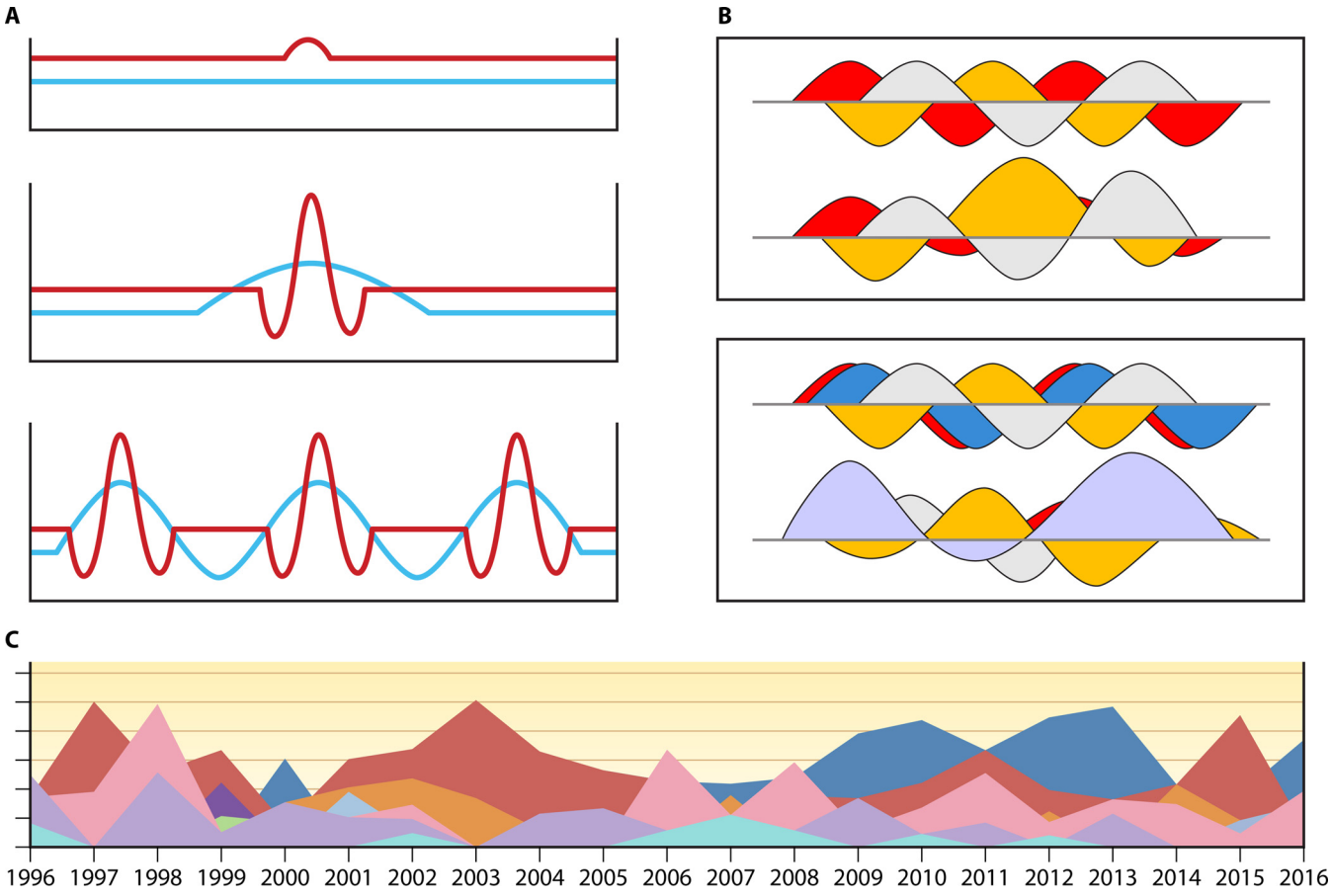


FIG 8 Populational (clonal) fluctuations and antibiotic resistance. (A) The equilibrium between the red and blue subpopulations is locally perturbed, occasionally due to the local antibiotic selection of a recently acquired resistance trait (or a local adaptive advantage), giving rise to wave dynamics recalling a Turing instability (634). The local selection of the red population influences the blue one, which might start competing with the red, creating an expansion of instability, giving rise to new fluctuations in the equilibrium of both the red and blue populations. (B) In the upper box, three populations or clones (colored red, yellow, and gray) fluctuate in a given environment (as the microbiota). Eventually the yellow population is selected, altering the other populations. (C) The simultaneous selection of the blue and red waves results in a merging, with the emergence of a new and predominant population, a superclone (635), as might occur in environments exposed to a variety of antibiotics. At the bottom, a real fluctuation pattern of *E. coli* clones along years in a single hospital (unpublished data). The main concept represented here is that antibiotics contribute to the instability of the clonal structure of bacterial populations, giving rise to dominant waves that can spread across the environment.

of the whole microbial consortium and its optimal equilibrium with the environment. To a certain extent, the community should coevolve with the resistant taxa.

Microbiotic ensembles frequently interact permanently with a particular environment, such as human and animal intestinal microbial communities, expressing a coevolutionary relationship. This interaction can be expressed as niche conservatism, the tendency for bacterial species to retain ancestral traits that ensure the species' original (selected, historical) functions within the microbial consortium (532). To maintain such homeostatic behavior in open environments, bacterial organisms might have cumulatively evolved traits that protect their interactive network (resilience traits), including those affecting AMR. These MGEs frequently correspond to populations that have been selected in the past by antibiotic exposure, in the same host or in a connectable host. In a sense, the MGEs in the community keeps a historical record of previous selective events. In case of reexposure, the MGE population employs a strategy like immunological memory in B cells and T cells in vertebrates; the "mechanism of resistance" is distributed by HGT among susceptible relatives. This strategy spares the need for harboring resistance genes, imposing certain fitness costs (including the cost of MGEs) on the host cell, which partly explains the fact that certain susceptible bacteria survive and that the curve of resistance prevalence in most susceptible species levels

off at a certain proportion. As previously stated, resistant bacteria might also protect the susceptible ones by providing genes and detoxifying the local antibiotic, in a cheater-altruist dynamic. If lateral gene transfer specifically protects phylogenetically close populations, detoxification protects the entire community, at least the spatially related, "granular" community.

Evolutionary Trajectories in Human Microbial Communities

The evolution of complex systems such as integrated microbial communities is slow compared with that of discrete populations, given that the interactive network provokes a high degree of robustness. The combination of *ex unibus plurum* (diversification, evolution) and *ex pluribus unum* (unification, robustness) processes (82) ensures the plasticity of microbial communities. A key point is the understanding that many microbial communities (such as the intestinal microbiota) should be reassembled from their components with high frequency (as in all sterile newborns) to the extent that the community replicates as a biological unit (48). The trajectories required to achieve the final integrated pattern might originate from different members pioneering the colonization process and establishing niche segregation colonization patterns (482, 533). The existence of community composition types (enterotypes) illustrates these differences (483). The initial microbiome composition determines its reshaping by antibiotics (534).

Patients intensively treated with antibiotics over decades are a source of resistant bacterial populations enriched in number by selection and consequently by host-to-host transmission. These resistant organisms overflow a patient's bacterial compartment to integrate the normal microbiota of healthy, nontreated individuals. Transmission of resistant organisms can occur from mothers to newborns, from treated patients to relatives (535), and in travelers exposed to other microbiotas (536).

As significant AMR becomes concentrated in certain populations of *Proteobacteria* and *Firmicutes*, conditions promoting their proportional increase in the intestine will augment AMR. These conditions include malnutrition (particularly in children and frequently associated with intestinal overgrowth), a high-fat diet, obesity, older age, and travel to areas with poor sanitation (537–540, 637). If, in the long term, resistant *Proteobacteria* and *Firmicutes* are consistently increased as components of the human microbiota, the entire microbial community is expected to evolve to explore novel equilibrium possibilities. The evolutionary and clinical consequences of such modifications (new equilibria) remain to be explored.

We cannot rule out the possibility that the community evolution of AMR might reach evolutionary stasis, either because antimicrobial agents are no longer required for treating infections (imagine a new era based on controlling the host response to bacterial challenges) or simply by the erosion of resistance fitness peaks. As stated before, once resistance and resilience reach a certain level in the normal microbiota, the selective effect of antibiotics should decrease.

Evolutionary Trajectories and Microbiota Community Coalescence

Over the last century (although the process started in the Neolithic period), communication among environmental, animal, and human microbiotas has been greatly facilitated by anthropogenic intervention because of increased environmental overlapping, the world homogenizing power of globalization, and the asymmetrical increase in the number of individuals in the planet's various biological species. Along with the increase in human population size, there has been a simultaneous increase in the population size of highly uniform types of food animals. For instance, the cattle inventory in 2018 was one billion head, and half of the world's stock of approximately 23 billion chickens are highly genetically homogeneous (by artificial selection of the most productive breeds) and are fed identically, thereby producing parallel increases in the microbial populations contained in their microbiota, consequently enhancing the possibility of merging human and animal microbiotas, known in ecology as "community coalescence" (541), frequently led by shuttle bacterial groups of generalist species (or clones within species) able to multiply in the microbiomes of various hosts, including

humans, animals, and plants (200). Coalescent microbiotas (the degree of coalescence will need to be measured in more detail in future research) also encompass free, natural environments. One consequence of gut colonization is the net increase in the density of resistant populations that are excreted into the environment. Resistome composition across habitats is generally structured by bacterial phylogeny along ecological gradients, with strong interactions between human populations and polluted environments, particularly in low-income habitats with poor excreta management strategies, particularly in areas of high antibiotic consumption (542, 543).

Antimicrobials and Sanitation Agents in the Environment

Ecological and evolutionary processes frequently operate on similar timescales (544). With the exception of resistance acquisition in pathogens by recombination with genes originating in commensal organisms sharing the same microbiota, the primary event of novel resistance acquisition is expected to occur not in clinical settings but in ecosystems where the environmental donor and the pathogenic receptor meet (545). The importance of anthropogenic antibiotic pollution in the environment is based on the selection of low-level, frequently unspecific mechanisms of resistance in a very large and heterogeneous ensemble of bacterial populations. Antibiotic resistomes, including MGEs, are significantly enlarged in periurban areas (546, 547).

Considering that microbial environments are highly complex and structured, some of their components might progress to higher fitness (resistance) peaks. Better tools are urgently needed for establishing the selective forces acting at microenvironmental (submillimeter) scales (548, 549). In natural environments, antibiotic-resistant populations and the communities hosting them are in close contact and interact with many other biological entities, such that changes in the biosphere and microbiosphere should have consequences in the distribution of antibiotic-resistant populations (6).

Biocide compounds, including disinfectants, antiseptics, heavy metals, food preservatives, and detergents, have been increasingly employed to reduce bacterial contamination. There are remarkable differences among antibiotic resistance and biocide activity levels (237). However, acquired, inheritable resistance to biocides remains rare, and the selection of AMR by biocides is infrequent (550). Interestingly, numerous biocide-resistant mutants have shown increased susceptibility to certain antibiotic compounds, which specifically act on cell envelopes such as the cell wall (beta-lactams) and cell membrane (poly-L-lysine, polymyxin B, colistin, and antimicrobial peptides). Biocide-resistant mutations are frequently found in genes that have a role in energy production, membrane biosynthesis, amino acid metabolism, and transport across cell envelopes (551, 552). As it is known, there is a strong connection between cell wall and membrane growth, determining the frequency of cell division (553).

Physical disinfection with UV irradiation is employed in water treatment plants. UV-light-emitting diodes are a useful tool for reducing bacterial loads without releasing disinfectant by-products; however, they require appropriate disposal facilities to prevent mercury release, potentially affecting the selection of metal-antibiotic-resistant bacteria. Among chemical disinfectants, chlorine is classically the most employed, but chlorination appears to have low (if any) effects on the evolution of antibiotic-resistant organisms.

Urban wastewater treatment plants might be considered one of the hot spots in the release of AMR into the environment (195, 554). A number of nonantimicrobial procedures have been classically applied to wastewater treatment plants (555). The application of membrane bioreactors, sequencing batch reactors, and activated sludge has significantly reduced the density of resistant populations in water, in contrast with biological filtering and upflow anaerobic sludge blanket technology (556). When anaerobic sequencing batch reactors were employed to treat pharmaceutical wastewater containing sulfamethoxazole, tetracycline, and erythromycin, multiresistant organisms were detected in the reactor's effluent; however, enriched ARGs frequently belong to nonpathogenic bacteria (557, 558).

Sewage treatment plants exert a powerful modifying force on the species composition of the incoming contaminated water, which influences the amount and type of

resistance genes, making the selective effects of antimicrobials in the effluent difficult to assess (559). However, meta-analyses have shown that composting and drying significantly reduce the relative abundance of resistance genes and MGEs in organic waste but only marginally in anaerobic digestion (560). The selection and evolution of AMR in soils are likely enhanced by common fertilization strategies (e.g., nitrogen fertilizers strongly affect the soil content of ARGs) (20). It is difficult to imagine decontamination procedures which might have deleterious ecological effects.

Antibiotic Resistance, Bacterial Communities, and Pathogenicity

We previously and extensively addressed the interplay of AMR and virulence in a review in this journal (561). AMR, virulence, transmission, and general bacterial fitness are closely linked processes, with a high degree of cross-epistasis and coevolution of the involved networks. Methods have recently been proposed to investigate such interactions from a systems biology perspective (562). However, the definitions of "virulence genes" and "pathogenicity genes" remain extremely confusing. To be pathogenic, the organism should be endowed with traits facilitating establishment in the host, and most so-called "virulence genes" encode colonization factors. Paradoxically, organisms less adapted to colonization might be more pathogenic, pushed to invade empty spaces out of the highly competitive areas where the normal microbiota is located. Given the long-term adaptation between hosts and microbiota, the most abundant bacteria in human or animal hosts are rarely the more virulent ones. Efficient colonizers have higher cell densities and wider access to genetic interactions, favoring the acquisition of AMR.

A number of examples in which AMR is associated with lower virulence are presented below. The constitutive hyperproduction of chromosomal AmpC beta-lactamase reduces bacterial fitness and virulence (563). Vancomycin-resistant *Enterococcus*, colistin-resistant *Acinetobacter* strains, and porin-deficient carbapenem-resistant or multidrug-resistant *P. aeruginosa* (with resistance in the last involving efflux pumps) are less virulent in animal models and frequently in the clinical setting (564, 565). In neonatal sepsis caused by $bla_{\text{NDM-1}}$-positive *Enterobacteriaceae*, mortality was lower (13.3%) than for cases caused by $bla_{\text{NDM-1}}$-negative organisms (22.2%) (566). Fluoroquinolone-resistant *E. coli* organisms tend to have fewer virulence factors than susceptible ones and are less pathogenic (567, 568). In the case of *Staphylococcus aureus*, there are no differences in clinical virulence between MRSA and methicillin-susceptible organisms; mupirocin resistance-conferring mutation of the *ileS* gene acts epistatically with other loci, reducing pathogenicity traits (569, 570).

Epidemics caused by multiple antibiotic-resistant clones ("high-risk clones") are, however, a major cause of morbidity and mortality, constituting a recognized worldwide public health problem, which appears to contradict the statements of the former paragraph. The main reason for this apparent paradox is that the selection of antibiotic-resistant populations through the use and release of antimicrobials increases the absolute density of resistant cells (571). By reducing the fitness of competitors, antibiotics act as a "colonization helper" of resistant populations. The outcome is an increased frequency of resistant populations, resulting in a number of consequences. First, the high frequency of resistant cells increases the ability for host-to-host transmission, particularly in hospitals and on farms. Second, the increased frequency favors the access of resistant cells to other bacterial populations, which are potential donors of new antibiotic resistance genes and virulence-colonization determinants. Third (and most importantly), the absolute density of resistant organisms in the gut increases the likelihood of invasion of the host's tissues; if resistant populations prevail, the probability of translocation and the risk of bacteremia by such organisms increase (572). Lastly, as invasive infections caused by resistant organisms increase, a larger number of novel antibiotics are employed, alone or in combination, favoring the evolution toward multiresistance.

PREDICTING EVOLUTIONARY TRAJECTORIES

The predictability of evolution depends on our knowledge of the fraction of the trajectories in fitness landscapes that are accessible for evolutionary exploration (573). In other words, predictability depends on our knowledge of the evolutionary constraints

influencing (in this case) the development of AMR (574). A frequently employed test for predicting mutational paths is the repeatability of evolutionary trajectories in replicate populations, which depends on the emergence rates of variants, their fitness effects, and their interactions (including epistasis). These experiments might be complemented by directed research, constructing site-specific mutagenesis genotypes with all single and combined mutations that are predicted to be under positive selection in phylogenetic analysis or evolution experiments and subjecting them to controlled selective environments (68). These empirical fitness landscapes (575) include exposure to different antibiotic concentrations and combinations or sequences of antimicrobials and changing nutritional or growth conditions. Experimental long-term evolution experiments have been instrumental in the exploration of adaptive pathways in bacteria.

Experimental Evolutionary Pathways

Long-term evolution experiments and historical contingency. Experimental evolution is the study of evolutionary processes occurring in experimental populations in response to conditions imposed by the experimenter (242). The hallmark of the studies on experimental evolution in microbiology is the famous long-term evolution experiment (LTEE) launched in early 1988 by Richard Lenski to test the repeatability of evolutionary dynamics across replicate populations (576–579). In this LTEE, 12 replicate *E. coli* populations were placed into tubes of minimal liquid medium containing glucose as the limiting resource. In this "from here to the eternity" experiment, 1% of each culture was seeded into fresh medium every day. Every 500 generations, the remainder of each population was frozen to keep a record of the accumulated changes for further studies. Currently, evolution to 80,000 generations in what appears to be a stable scenario (only population fluctuations in seeding a new tube each day) has been achieved. The study highlighted that evolution, even in a simple scenario, is an intriguing mix of random (mutation and drift) and directional (natural selection) processes. The generation of mutants might produce negative genetic interactions offering a possibility for new beneficial mutations to emerge (239). In general, these studies show the never-ending history of bacterial evolution: after so many generations in a constant environment, there are sustained fitness gains in variability, implying that both adaptation and divergence can continue, possibly indefinitely (580), a lesson that applies to AMR. The Shakespearian-Huxleyian question to apply here is whether "evolutionary time will reach an end."

Parallel evolution can be shown in the LTEE. The fitness of *E. coli* in extracting all possibilities from the culture medium (and the products released by bacterial metabolism) increased during the experiment, and the trajectories of changes explaining these gains were similar across the replicate populations but not identical. Mutations were consistently fixed in a number of genes in all 12 populations, although the exact mutations at the sequence level differ in almost every case. A parallel evolution among replicates in gene expression was also demonstrated, due to parallel changes in a gene encoding a "global" regulon. However, divergent evolution was also detectable. For instance, the emergence of the ability to use citrate occurred in only one of the replicates; some of the lines evolved the inability to use ribose (581).

The citrate-using variant emerged only after 31,500 generations, as a random, fortuitous "historical contingency." The expected frequency for such a variant is less than 3×10^{13} per cell and generation (582). A genetic prehistory of the ability to use citrate was detected in three coexisting clades (within the same replicate) that evolved a tandem duplication, increasing the expression of a previously silent citrate transporter (583, 584). However, only one of the three clades developed a significant citrate-using phenotype, indicating the need for further changes allowing expression.

Similar "historical contingencies" are expected in AMR trajectories. Bacterial populations, with huge population sizes, are spread in a vast variety of changing environments, and therefore, the accumulated "time-history" of bacterial lineages is extremely high. As in the case of citrate utilization in the LTEE, silent mutations might arise by historical contingencies that eventually facilitate the emergence of significant AMR. These accidents will determine or produce the extinction of particular evolutionary trajectories. Given the random nature of

most environmental changes, however, trajectories, at least in these initial stages, will remain largely sensitive to history and are therefore unpredictable (585, 586), thus precluding the "replaying of the tape" of evolution. Of course, nature differs from test LTEE identical tubes; in the case of AMR, microorganisms with an identical ancestor are be placed in multiple environmental circumstances but subjected to the same selective force; previous adaptative events dictate the trajectories of later evolutionary processes (587).

The desirable combination of the LTEE and realistic empirical fitness landscapes is still far from our technical capabilities, given that this combination implies "sequential replication of landscapes." However, the approach provided by Kishony's group (588) in which bacteria spread and evolve against a large antibiotic gradient in soft agar megaplates is promising in this respect. Empirical fitness landscape studies are based on the artificial mix of various mutations that presumably influence fitness (such as AMR) and on studying the fitness of these genotypes and their epistatic combinations (589). The main problem is sampling to detect all variants present in the local population, across the gradient, with which to perform independent fitness studies. However, a reasonably good resolution of these fitness landscapes might be obtained in the analysis of areas exposed to strong selection (590).

A turn in modeling complexity results from the need to merge different selective fitness landscapes, changing the selective environments. This approach might be achieved by "two-step" evolution experiments, starting with an LTEE in a particular environment. The subsequent replicate populations are then transferred and propagated for a new LTEE in a new environment (591). This has a clear application for AMR evolution (e.g., for detecting changes leading to multiresistance and antagonistic pleiotropy). Environmental changes might produce evolutionary constraints and trade-offs (correlated changes move in opposite adaptive directions), which might create conflicts between the survival and reproduction components of fitness.

Advances have been made during the past decade in constructing various types of miniaturized, automated fitness monitoring applications for *in vitro* evolution (i.e., evolution machines). Particularly promising are the applications that take advantage of microfluidic technology, creating "microfluidic landscapes" (592). Combined with live-cell imaging, microfluidics can help address the issues regarding the relationships of physiological phenotypical adaptation, selection, and inheritable resistance across fitness landscapes.

Directed experimental evolution of resistance. Directed experimental evolution experiments are those that are designed to evaluate the possibility of obtaining successive best-fit antibiotic-resistant variants under controlled exposure to antibiotics. These experiments are frequently based on serial passages of a culture containing the ancestor(s) population, thus ensuring bottlenecks in the daily propagation of a sample from one culture tube to the next. The successive culture tubes typically contain growing antibiotic concentrations, and the objectives are to obtain the variant with the highest MIC and to explore the mutational path that has produced such a variant. On other occasions, the goal of experimental evolution is to ascertain the possibility of obtaining variants that broaden the spectrum of antibiotic inactivation. As previously stated, an efficient method is to identify (by genetic analysis) altered positions in the sequence of the resistance gene that have likely been subjected to antibiotic positive selection, to construct these mutants and their combinations by site-directed mutagenesis, and to sequentially expose the corresponding cultures to increased concentrations of the various antibiotics. These studies frequently reveal the possibility of diversification into several possible pathways (68, 593). Direct genetic reconstruction of available trajectories might consider not only AMR but also compensatory evolution and enzyme stability (594).

Experimental evolution of fitness costs of resistance. AMR usually comes at variable fitness costs, since resistance mutations typically target important biological processes in the cell, whereas the acquisition of resistance via HGT is typically associated with the costs imposed by MGEs (308, 595). Quantifying fitness costs and their

reversibility is critical to predicting the resistance determinants most likely to succeed and to affect evolutionary trajectories (596). Several techniques are available to measure fitness costs. The simplest method is to measure the growth rates of resistant and ancestral clones by monitoring optical density during growth as monocultures (often employing multiwell adapted spectrophotometers). Growth rates can be employed as a proxy for fitness; however, this method is not overly sensitive to small differences in fitness. More accurate estimates of fitness can be obtained by performing pairwise competition experiments, which measure the change in the ratio of two strains after growth as mixed cultures (597). Competition experiments are preferred over single-culture techniques because they integrate several growth parameters, such as lag phase, growth rates, and efficiency of resource usage (596, 597). New computational methods have been proposed to predict the outcome of competition experiments from growth curve data (598). Competition experiments can be performed in test tubes or *in vivo* by infecting model animals with a mixed bacterial culture, which is likely to provide a more realistic view of the competitive ability of AMR mutants.

However, the cost of AMR is itself an evolvable trait. Bacteria readily acquire secondary mutations that alleviate the fitness costs associated with resistance. This process, known as compensatory evolution, can be reproduced under laboratory conditions. Most experimental designs consist of propagating resistant bacterial clones during a relatively large number of generations while frequently monitoring for fitness gains using the above-described methods. Propagation is typically performed by cycles of dilution and growth (serial passages) of the selected bacteria, either in the presence of the antibiotic to which the test clones are resistant or in antibiotic-free media. Alternatively, continuous growth of the microorganisms can be achieved using chemostats and bioreactors, in which a constant supply of nutrients is provided to allow the microorganisms to grow steadily (599). Although *in vitro* compensatory evolution experiments have provided invaluable insights into the evolution of AMR, evolutionary trajectories crucially depend on the environment. Compensatory evolution experiments performed *in vivo* often lead to different results than those from experiments performed with test tubes (222).

Directionality and Repeatability of Evolutionary Trajectories

Based on the notion of contingency (the impossibility of determining whether something is either true or false under every possible evaluation) and using primitive computer modeling, the paleontologist Stephen Jay Gould expressed in 1990 his deep concerns about the repeatability of evolutionary trajectories and outcomes by "replaying the tape of life" (600). Since then, the contingency-convergency debate has remained central in evolutionary biology. Noncontingency occurs (e.g., in developmental genetics), and conserving a complex solution to an adaptive problem is frequently simpler than repeatedly reinventing the solution (the "if it ain't broke, don't fix it" maxim) (601), which applies to evolutionary trajectories in antimicrobial resistance, at least for bacterial organisms of the same lineage (the "inventor"). It is possible that different evolutionary trajectories might recruit variant steps or insert functionally equivalent changes without altering the final phenotype or even that different trajectories produce identical results (convergence). The reality of convergent evolution suggests that iterated evolutionary outcomes might be identified because they follow a seemingly law-regulated determination (602). However, numerous studies have revealed the surprising result that developmental pathways do in fact diverge throughout time, even with no accompanying change in the phenotypic outcome. Very close trajectories at the start of the process are expected to rapidly diverge, given that divergence is exponential (603). However, predictability of evolution depends nonmonotonically on population size (604).

Whole-genome sequencing has recently been employed to study the reproducibility of adaptive trajectories. In general, adaptive convergence explains the increased reproducibility of the advanced steps in the trajectory once a favorable phenotype (not necessarily a fixed constellation of mutations) is obtained (605). Assessment of potentially advantageous phenotypes can be obtained by experimental fitness assays, including the study of substrate-binding affinities of mutant proteins (606). Results

from genetic reconstruction experiments indicate the predictability of the associations between antibiotic resistance chromosomal mutations and fitness and suggest that epistatic effects are rare even when up to four mutations are combined (246).

Modeling Evolutionary Processes in Antibiotic Resistance

Mathematical models. A wealth of mathematical models has been developed to study the evolution of AMR. The classic studies mostly conducted in the early 1990s (607) were "compartmental models." A human host population is typically compartmentalized into susceptible and colonized hosts (with susceptible and resistant bacteria). The frequencies of susceptible or colonized hosts are dependent on their densities, being modified by therapy (use of antibiotics, dosages and therapeutical schedules, and pharmacodynamics and pharmacokinetics), prevention of transmission, and natural clearance of bacteria, including the immune response. These frequencies are measured by applying deterministic models based on a system of ordinary differential equations describing the dynamics of the densities of each type of host and the susceptible and resistant populations. Stochastic models are in most cases agent based, in which the hosts and bacteria are tracked individually, based on the individual probability of a host being colonized by a susceptible or resistant bacterium. These models frequently employ the Monte Carlo protocol, calculating the daily probability of moving from one compartment to the other (608, 609). Such compartmental models can be "interhost" models, applied to the transmission of resistance between hosts, such as in the spread of resistance in hospitals or on farms, and "intrahost" models, designed to predict the emergence of resistance within the treated host (609). Similar models have been applied to study more basic problems of AMR, such as the horizontal transfer of resistance genes in bacteria (610). To a certain extent, mathematical approaches have been instrumental to obtain estimated "evolutionary rates," considering base substitutions through comparative studies of nucleotide sequences and the derived phylogenetic analysis (611). Other mathematical modeling studies are based only on the "possible" structural landscapes of molecules, taking RNAs or protein molecules as variable "evolutionary units" and "fitness of phenotypes" as the replicative or enzymatic activities or their stability (247). These modeling studies do not encompass all of the complex steps and interactions of evolutionary processes, however, and require severe reductionism to be able to describe (with deterministic and stochastic modeling combinations) certain traits of the processes under study (612).

Synthetic biology modeling evolutionary trajectories. "Long-term behavior is unpredictable" (613). Natural complex systems increase in complexity over time, and natural complex bacterial systems (from communities of microorganisms to communities of genes), such as those involved in the evolution of AMR, have much higher robustness to perturbations than engineered communities. Synthetic biology offers appropriate tools for the desirable reduction in the complexity factors influencing evolutionary trajectories. By developing genetic parts and devices based on transcriptional, translational, and posttranslational modules, numerous genetic circuits and metabolic or AMR pathways can be programmed in single cells, including those with a reduced genome (chassis) (614). Synthetic biology offers a rich potential for engineering microbial consortia (615) and, in general, natural and synthetic microbial ecosystems (616, 617). Until recently, synthetic regulatory networks have been designed manually; however, this limit has been surpassed with the development of genetic circuit design automation, in which dozens of circuits can be tested in living cells, including numerous types of adaptive responses (transcriptional factors, RNA-based regulation, protein-protein interactions, and effects of recombinases) (618).

Network analysis of evolutionary trajectories. Classic phylogenetic trees have been extensively employed to represent evolutionary processes and might clarify the historical succession (pathway) of mutational events giving rise (in the case under treatment) to a

particular DNA or protein sequence involved in AMR. In simple cases, genealogical trees predict trajectories. In more complex ones, automated phylogenetic tools have been applied to reconstruct ancestral sequences and explore mutational trajectories (73). Inspired by bioprocess engineering, a modeling framework based on flux-balance analysis has been proposed as a mathematical method for simulating the construction of metabolism networks (619), a method that could eventually be applied to AMR.

However, a single genealogical tree can no longer represent the complexity of evolutionary trajectories. Trees are embedded into networks (Fig. 7). The complexity produced by lateral gene-diverging transfer among members of different lineages and introgressive merging events (44) in which elements of various evolutionary units at different biological hierarchies, and particularly among kin evolutionary units (43), interact and coevolve as composite objects requires considering "linked-trees woods" or multidimensional supertrees, which should be constructed with networks.

Sequence similarity networks offer appropriate images of genetic diversity. However, these images do not explain the differences in similarity and the causes for divergence. A multiplicity of network analysis tools are now available which can automatically identify composite objects formed by genetic fragments with distinct evolutionary histories (384), representing them (with a quantitative dimension) and formally producing comparisons among an extensive number of sequences-objects. In terms of exploring the evolution of AMR, we can deduce the possible positive or negative interactions between elements (from genes to communities and on both horizontal and vertical axes) affecting potential evolutionary trajectories. Network analysis offers the opportunity for understanding why some evolutionary entities rarely merge or exchange traits (they can be closely related upon transmission events but without structuring a consistent association) or why others easily share common public goods. Network analysis refers to the antibiotic resistance gene-coexistence interactions of the genes with the host bacterial genes, between-MGE interactions and relations with the host cell, and the building up of microbial communities.

For these purposes, bipartite graphs are adequate because they allow heterogeneous biological entities (e.g., plasmids and bacterial hosts) to be connected by edges (relations) (77). This type of analysis has revealed that a multitude of gene families are shared by means of MGEs in different bacterial groups, including ultrasmall bacteria (620). The analysis has enabled the identification of the "circles of species" that can be contaminated with ARGs and of the commonality of the components of these circles that will be detected by techniques such as contact metagenomics (204).

This progress in the network analysis of evolutionary trajectories has provided a new image of evolutionary trajectories (621–623), a rapidly growing field that now encompasses multilevel, transhierarchical networks that consider the evolutionary and mechanistic relations shaping phenotype-genotype maps (624, 625).

Computational modeling of multilevel antibiotic resistance. AMR evolutionary trajectories involve individual entities across various hierarchical levels, from DNA fragments to microbial communities (54). There is a need for integrating intrahost and interhost modeling to address the evolutionary epidemiology of AMR (609). Based on bioinspired natural or cellular computing, membrane computing (626) has recently made advances in the multilevel analysis of AMR (627). The various actors of nested biological scenarios are represented particular entities surrounded by "membranes" (objects, "individualized" by membranes, from genes to MGEs, species, bacterial communities, and hospitals). Thus, a membrane can be located inside another membrane of a higher hierarchy. Membranes are endowed with "rules" ensuring interactions with other membranes across hierarchies to independently replicate, propagate, become extinct, transfer into other membranes, exchange information according to flexible rules, mutate, and be selected by external agents (628). Membrane computing enables us to dissect the influence of changes in any evolutionary unit at a particular hierarchical level on the outcome of the entire system (for instance, how

TABLE 1 The components shaping pathways and trajectories in the evolution of antibiotic resistance[a]

Component type	Components
Evolutionary objects	Antibiotic molecular targets, antibiotic transporters, single and supraprotein domains, rRNA sequences, intrinsic resistance genes, regulators of antibiotic transporters and resistance genes, stress response networks, acquired AbR genes, noncoding segments of genome, random chromosomal sequences, genes with epistatic relations with AbR, contingency loci, operons, insertion sequences, small intergenic repetitive sequences, gene cassettes, integrons, transposons, plasmids, integrative-conjugative elements, genetic islands, bacteriophages, bacterial species, bacterial subspecies, bacterial clones (genomotypes, STs), clonal ensembles, genetic exchange communities, metagenomotypes (i.e., enterotypes), resistomes (intrinsic and mobile)
Evolutionary processes	Growth, mutation, genetic diversification, epigenetic epistasis, fitness cost and cost compensation, gene amplification, gene conversion, gene redundancy, gene promiscuity by HGT, gene recombination, gene insertions and deletions, gene silencing, gene degeneracy, gene decontextualization by HGT, promoter recombination, genome recombination, gene conjugation, gene transformation, gene transduction, transfer by extracellular vesicles or nanotubes, MGE transmission, MGE-host interactions, MGE mobilization, MGE recombination, MGE copy no., MGE maintenance, MGE incompatibility, bacterium-bacterium contacts and recognition, bacterial antagonism and cooperation, interhost transmission, host-bacterium interactions, microbiota coalescence
Evolutionary mechanisms	Selection by other reasons than AbR, selection dependent on AbR, cross-selection, coselection, selection in antibiotic gradients, random drift, random draft, gene hitchhiking, neofunctionalization-exaptation, founder effects, persistence, tolerance, inducibility of AbR, resilience in the presence of Ab, changes in fitness, niche exploitation and coexploitation, niche construction, habitat compartmentalization, spatial structuration, transmission, dispersal, clonal shifts, clonal waves, clonal bunch selection, reticulation of evolutionary trajectories
Evolutionary drivers	Bacterial stress, bacterial bottlenecks, human antibiotic consumption, animal antibiotic consumption, agricultural antibiotic consumption, historical antibiotic use, antibiotic pharmacokinetics/dynamics, collateral susceptibility and resistance, human and animal factors (age, health, and nutrition), bacterial transmission, hygiene, sanitation, crowded human or animal populations, water and sludge reuse, antibiotics and biocides in the environment, pollution with heavy metals, environmental pollution with human and animal bacteria, decrease in animal and global biodiversity, environmental variation, global warming, social norms for the use of antibiotics, social norms for environmental health

[a]Evolutionary objects are the biological substrates, from proteins to microbiotas, on which evolutionary processes act, producing phenotypes, whose frequency is governed by evolutionary mechanisms, which are under the influence of evolutionary drivers. MGE, mobile genetic elements; AbR, antibiotic resistance.

the plasmid conjugation rate or cellular cost compensation of harboring plasmids influences AMR in a hospital) (627).

ANTHROPOGENIC EFFECTS ON THE MICROBIOSPHERE AS A SOURCE OF UNCERTAINTY

Microbial pathways and trajectories involving AMR occur in a multiparametric changing biological world (629) (Table 1) influenced by anthropogenic activities, resulting in the reduction of the diversity of certain species but likely also fostering speciation (449, 630). Humans create and select environments, and there is a reciprocal selection between biological entities and environments. Anthropogenic action on environments produces effects on AMR pathways and trajectories, with pathways the more rigid parts of the evolutionary trajectories. In a previous review (82), the intrinsic indetermination of evolutionary trajectories was compared with the dynamics of a multiple pendulum/oscillator (631) as shown in Fig. 9. How do we make intelligible the indetermination? Should we accept the impossibility of knowing AMR trajectories?

Knowing the limits of our endeavor to discover laws of the natural world is an obligation of science. Our knowledge and the possibility of communicating our findings to future generations depend on the rational structure of our proposals. Rationality requires the existence of a certain order in the interaction among the elements involved in the process under study or at least some solid probabilistic associations; chaos cannot be explained. In this review, we have examined the plethora of processes involved in AMR. Evolutionary pathways are composed of logical sequences of events in the acquisition of resistance and, despite their diversity, can frequently be faithfully reproduced in controlled evolutionary experiments. However, evolutionary trajectories depend on an unlimited number of stochastic events influencing myriad interactions among a hierarchy of nested biological elements, from proteins to genes to

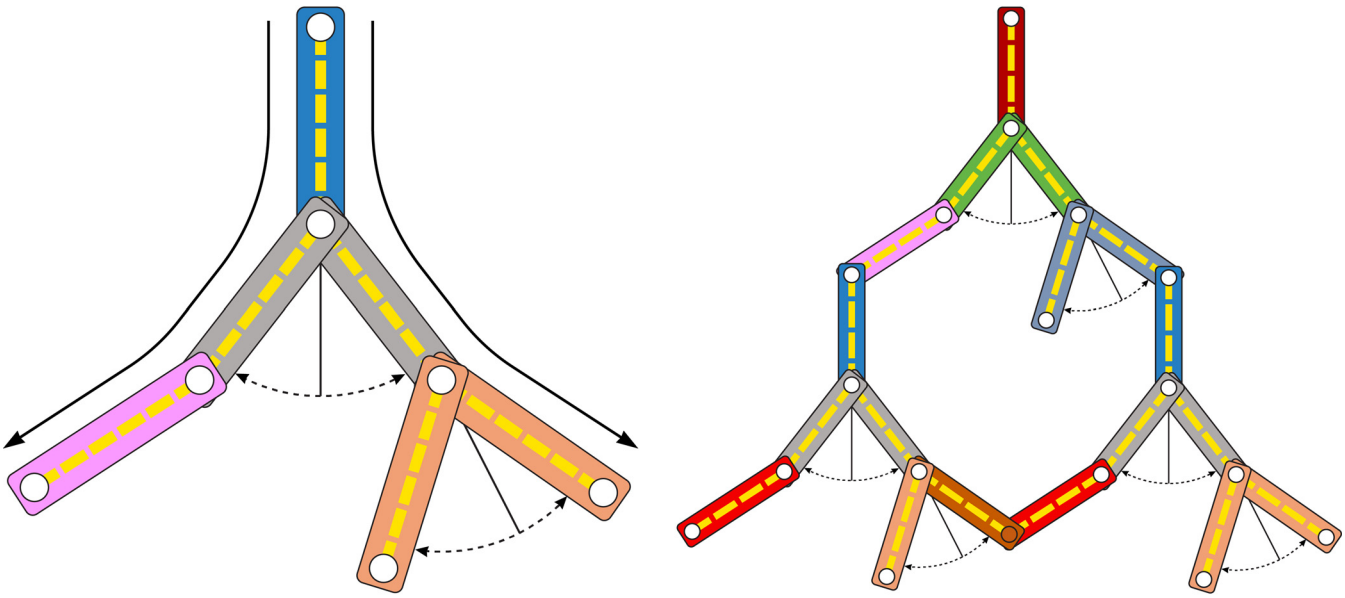


FIG 9 Evolutionary pathways and trajectories. On the left, adaptive pathways and trajectories are represented as parts of a multibody pendulum, with mobile rigid members hinging on each other. The rigid parts represent the pathways, formed by broken yellow elements, corresponding to series of successive events (as mutations) leading to an efficient resistance phenotype, which are predictable and reproducible to a certain extent in the laboratory. However, these rigid parts located in bacteria oscillate by moving in different environments, where they can approach and be linked by swivel joints (white circles, representing mobile genetic linkages) to other organisms. An immense number of possible trajectories are thereby created, each offering new possibilities for interaction and linkage with other rigid parts, again eventually mediated by mobile genetic elements (the ball joints). The resulting multiple pendula greatly increases the indetermination of trajectories, approximating a chaotic behavior, with diversifying kinetics (black arrows). On the right, the possibility of loop formation among the trajectories is presented, providing a certain rigidity (and thus potential predictability) to the system. Note that the rigid parts might correspond to various units of selection, organisms, supraorganisms (such as species), and suborganisms (such as plasmids), which create a highly complex evolutionary frame.

species and communities, each acting in a selfish manner and all running on their own evolutionary trajectories and colliding and collaborating with other evolutionary trajectories. In the case of complex evolutionary trajectories, certain trends can be assumed by accurate and long-term observations; however, such trends only apply for shorts periods (as with weather prediction, which also deals with highly complex systems). As in the rest of the biological sciences, our understanding and our intelligibility of the evolution of AMR have a part that is logical, demonstrable, and based on solid information and a part that is based on undetermined information, which we can attempt to predict based on observations. Therefore, the knowledge of evolution is composed by thinkable and only showable parts, and a strategy of half-thinking, half-seeing is needed to make intelligible the evolutionary processes, including AMR (632). We are obliged to continue our daily tasks to ascertain the details of the multihierarchical interactions among entities involved in AMR, in the hope that in the future, complex computational models and artificial intelligence tools can help push the frontiers of our knowledge, to understand and control the negative influence of AMR on medicine, One Health, and Global Health.

ACKNOWLEDGMENTS

F.B. and T.M.C. were supported by grants funded by the Joint Programming Initiative in Antimicrobial Resistance (JPIAMR Third call, STARCS, ST131TS Programmes, JPIAMR2016-AC16/00039), the Carlos III Health Institute (ISCIII) of Spain/Ministry of Economy and Competitiveness, and the European Development Regional Fund A Way to Achieve Europe (ERDF) for cofounding the Spanish R&D National Plan Estatal de I+D+ i 2013-2016 (PI18/1942), CIBERESP (CIBER in Epidemiology and Public Health; CB06/02/0053), the Regional Government of Madrid (InGeMICS- B2017/BMD-3691), and the Ramón Areces Foundation. R.C. was funded by the Carlos III Health Institute of Spain, Plan Estatal DE IþDþI 2013–2016 (REIPI RD12/0015/0004 and RD16/0016/0011, Spanish Network for Research in Infectious Diseases, and projects PI15-00466 and PI19/01043) and cofinanced by

the European Development Regional Fund A Way to Achieve Europe (FEDER). J.C.G. was funded by the PI12/00567 from ISCIII.

We are all deeply indebted to Carmen de la Vega Losada, our wonderful project manager at the Department of Microbiology, for highly professional editing and formatting of our manuscript.

REFERENCES

1. Davies J, Davies D. 2010. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74:417–433. https://doi.org/10.1128/MMBR.00016-10.
2. MacLean RC, Millan AS. 2019. The evolution of antibiotic resistance. Science 365:1082–1083. https://doi.org/10.1126/science.aax3879.
3. Baquero F, Blázquez J. 1997. Evolution of antibiotic resistance. Trends Ecol Evol 12:482–487. https://doi.org/10.1016/s0169-5347(97)01223-8.
4. Baquero F, Canton R. 2017. Evolutionary biology of drug resistance, p 9–32. In Mayers DL, Sobel JD, Ouellette M, Kaye KS, Marchaim D (ed), Antimicrobial drug resistance, vol 1. Mechanisms of drug resistance. Springer International Publishing, New York, NY.
5. Ariew A. 2003. Ernst Mayr's "ultimate/proximate" distinction reconsidered and reconstructed. Biol Philos 18:553–565. https://doi.org/10.1023/A:1025565119032.
6. Hiltunen T, Virta M, Anna-Liisa L. 2017. Antibiotic resistance in the wild: an ecoevolutionary perspective. Philos Trans R Soc Lond B Biol Sci 372:20160039. https://doi.org/10.1098/rstb.2016.0039.
7. Sugihara G, May R, Ye H, Hsieh CH, Deyle E, Fogarty M, Munch S. 2012. Detecting causality in complex ecosystems. Science 338:496–500. https://doi.org/10.1126/science.1227079.
8. Koonin EV. 2012. The logic of chance: the nature and origin of biological evolution. FT Press Science, Upper Saddle River, NJ.
9. Mouton JW, Brown DFJ, Apfalter P, Cantón R, Giske CG, Ivanova M, MacGowan AP, Rodloff A, Soussy CJ, Steinbakk M, Kahlmeter G. 2012. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. Clin Microbiol Infect 18:E37–E45. https://doi.org/10.1111/j.1469-0691.2011.03752.x.
10. Simjee S, Silley P, Werling HO, Bywater R. 2008. Potential confusion regarding the term "resistance" in epidemiological surveys. J Antimicrob Chemother 61:228–229. https://doi.org/10.1093/jac/dkm423.
11. Kahlmeter G, Brown DFJ, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, Rodloff A, Steinbakk M, Urbaskova P, Vatopoulos A. 2003. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother 52:145–148. https://doi.org/10.1093/jac/dkg312.
12. Martínez JL, Coque TM, Baquero F. 2015. What is a resistance gene? Ranking risk in resistomes. Nat Rev Microbiol 13:116–123. https://doi.org/10.1038/nrmicro3399.
13. Andersson DI. 2015. Improving predictions of the risk of resistance development against new and old antibiotics. Clin Microbiol Infect 21:894–898. https://doi.org/10.1016/j.cmi.2015.05.012.
14. Martínez JL, Baquero F, Andersson DI. 2011. Beyond serial passages: new methods for predicting the emergence of resistance to novel antibiotics. Curr Opin Pharmacol 11:439–445. https://doi.org/10.1016/j.coph.2011.07.005.
15. Martínez JL, Baquero F, Andersson DI. 2007. Predicting antibiotic resistance. Nat Rev Microbiol 5:958–965. https://doi.org/10.1038/nrmicro1796.
16. Wright GD. 2010. The antibiotic resistome. Expert Opin Drug Discov 5:779–788. https://doi.org/10.1517/17460441.2010.497535.
17. Perry JA, Wright GD. 2013. The antibiotic resistance "mobilome": searching for the link between environment and clinic. Front Microbiol 4:138. https://doi.org/10.3389/fmicb.2013.00138.
18. Munck C, Albertsen M, Telke A, Ellabaan M, Nielsen PH, Sommer MOA. 2015. Limited dissemination of the wastewater treatment plant core resistome. Nat Commun 6:8452. https://doi.org/10.1038/ncomms9452.
19. Yang Z, Guo Z, Qiu C, Li Y, Feng X, Liu Y, Zhang Y, Pang P, Wang P, Zhou Q, Han L, Dai W. 2016. Preliminary analysis showed country-specific gut resistome based on 1267 feces samples. Gene 581:178–182. https://doi.org/10.1016/j.gene.2016.01.043.
20. D'Costa VM, McGrann KM, Hughes DW, Wright GD. 2006. Sampling the antibiotic resistome. Science 311:374–377. https://doi.org/10.1126/science.1120800.
21. Forsberg KJ, Patel S, Gibson MK, Lauber CL, Knight R, Fierer N, Dantas G. 2014. Bacterial phylogeny structures soil resistomes across habitats. Nature 509:612–616. https://doi.org/10.1038/nature13377.
22. Alvarez-Ortega C, Wiegand I, Olivares J, Hancock REW, Martínez JL. 2010. Genetic determinants involved in the susceptibility of Pseudomonas aeruginosa to beta-lactam antibiotics. Antimicrob Agents Chemother 54:4159–4167. https://doi.org/10.1128/AAC.00257-10.
23. Spanogiannopoulos P, Waglechner N, Koteva K, Wright GD. 2014. A rifamycin inactivating phosphotransferase family shared by environmental and pathogenic bacteria. Proc Natl Acad Sci U S A 111:7102–7107. https://doi.org/10.1073/pnas.1402358111.
24. Baquero F, Lanza VF, Cantón R, Coque TM. 2015. Public health evolutionary biology of antimicrobial resistance: priorities for intervention. Evol Appl 8:223–239. https://doi.org/10.1111/eva.12235.
25. Laskaris P, Tolba S, Calvo-Bado L, Wellington EM, Wellington L. 2010. Coevolution of antibiotic production and counter-resistance in soil bacteria. Environ Microbiol 12:783–796. https://doi.org/10.1111/j.1462-2920.2009.02125.x.
26. Wright GD. 2007. The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 5:175–186. https://doi.org/10.1038/nrmicro1614.
27. Christaki E, Marcou M, Tofarides A. 2020. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. J Mol Evol 88:26–40. https://doi.org/10.1007/s00239-019-09914-3.
28. Van Goethem MW, Pierneef R, Bezuidt OKI, Van De Peer Y, Cowan DA, Makhalanyane TP. 2018. A reservoir of "historical" antibiotic resistance genes in remote pristine Antarctic soils. Microbiome 6:40. https://doi.org/10.1186/s40168-018-0424-5.
29. Ninio J. 2010. Frail hypotheses in evolutionary biology. PLoS Genet 6:e1001067. https://doi.org/10.1371/journal.pgen.1001067.
30. Brosius J, Gould SJ. 1992. On "genomenclature": a comprehensive (and respectful) taxonomy for pseudogenes and other "junk DNA." Proc Natl Acad Sci U S A 89:10706–10710. https://doi.org/10.1073/pnas.89.22.10706.
31. Martínez JL. 2011. Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens. Front Microbiol 2:265. https://doi.org/10.3389/fmicb.2011.00265.
32. Fondi M, Karkman A, Tamminen MV, Bosi E, Virta M, Fani R, Alm E, McInerney JO. 2016. "Every gene is everywhere but the environment selects": global geolocalization of gene sharing in environmental samples through network analysis. Genome Biol Evol 8:1388–1400. https://doi.org/10.1093/gbe/evw077.
33. Carfi A, Pares S, Duée E, Galleni M, Duez C, Frère JM, Dideberg O. 1995. The 3-D structure of a zinc metallo-beta-lactamase from Bacillus cereus reveals a new type of protein fold. EMBO J 14:4914–4921. https://doi.org/10.1002/j.1460-2075.1995.tb00174.x.
34. Ferretti JJ, Gilmore KS, Courvalin P. 1986. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. J Bacteriol 167:631–638. https://doi.org/10.1128/jb.167.2.631-638.1986.
35. Long M, Vankuren NW, Chen S, Vibranovski MD. 2013. New gene evolution: little did we know. Annu Rev Genet 47:307–333. https://doi.org/10.1146/annurev-genet-111212-133301.
36. Yona AH, Alm EJ, Gore J. 2018. Random sequences rapidly evolve into de novo promoters. Nat Commun 9:1530. https://doi.org/10.1038/s41467-018-04026-w.
37. Song HS, Renslow RS, Fredrickson JK, Lindemann SR. 2015. Integrating ecological and engineering concepts of resilience in microbial communities. Front Microbiol 6:1298. https://doi.org/10.3389/fmicb.2015.01298.
38. Martinez JL, Sánchez MB, Martínez-Solano L, Hernandez A, Garmendia L, Fajardo A, Alvarez-Ortega C. 2009. Functional role of bacterial multidrug

efflux pumps in microbial natural ecosystems. FEMS Microbiol Rev 33:430–449. https://doi.org/10.1111/j.1574-6976.2008.00157.x.
39. Piddock LJV. 2006. Multidrug-resistance efflux pumps—not just for resistance. Nat Rev Microbiol 4:629–636. https://doi.org/10.1038/nrmicro1464.
40. Thiemann S, Smit N, Strowig T. 2016. Antibiotics and the intestinal microbiome: individual responses, resilience of the ecosystem, and the susceptibility to infections. Curr Top Microbiol Immunol 398:123–146. https://doi.org/10.1007/82_2016_504.
41. Morosini MI, Negri MC, Shoichet B, Baquero MR, Baquero F, Blazquez J. 1998. An extended-spectrum AmpC-type beta-lactamase obtained by in vitro antibiotic selection. FEMS Microbiol Lett 165:85–90. https://doi.org/10.1111/j.1574-6968.1998.tb13131.x.
42. Sanz-García F, Hernando-Amado S, Martínez JL. 2018. Mutational evolution of *Pseudomonas aeruginosa* resistance to ribosome-targeting antibiotics. Front Genet 9:451. https://doi.org/10.3389/fgene.2018.00451.
43. Stern DL, Orgogozo V. 2009. Is genetic evolution predictable? Science 323:746–751. https://doi.org/10.1126/science.1158997.
44. Bapteste E. 2014. The origins of microbial adaptations: how introgressive descent, egalitarian evolutionary transitions and expanded kin selection shape the network of life. Front Microbiol 5:83. https://doi.org/10.3389/fmicb.2014.00083.
45. Bapteste E, Lopez P, Bouchard F, Baquero F, McInerney JO, Burian RM. 2012. Evolutionary analyses of non-genealogical bonds produced by introgressive descent. Proc Natl Acad Sci U S A 109:18266–18272. https://doi.org/10.1073/pnas.1206541109.
46. Böttcher T. 2018. From molecules to life: quantifying the complexity of chemical and biological systems in the universe. J Mol Evol 86:1–10. https://doi.org/10.1007/s00239-017-9824-6.
47. Gould SJ. 2002. The structure of evolutionary theory. Harvard University Press, Cambridge, MA.
48. Gould SJ, Lloyd EA. 1999. Individuality and adaptation across levels of selection: how shall we name and generalize the unit of Darwinism? Proc Natl Acad Sci U S A 96:11904–11909. https://doi.org/10.1073/pnas.96.21.11904.
49. Baquero F. 2014. Genetic hyper-codes and multidimensional Darwinism: replication modes and codes in evolutionary individuals of the bacterial world, p 165–180. In Trueba G (ed), Why does evolution matter? The importance of understanding evolution. Cambridge Scholars Publishing, Newcastle upon Tyne, United Kingdom.
50. Ereshefsky M, Pedroso M. 2015. Rethinking evolutionary individuality. Proc Natl Acad Sci U S A 112:10126–10132. https://doi.org/10.1073/pnas.1421377112.
51. Ghaly TM, Gillings MR. 2018. Mobile DNAs as ecologically and evolutionarily independent units of life. Trends Microbiol 26:904–912. https://doi.org/10.1016/j.tim.2018.05.008.
52. Borges RM. 2017. Co-niche construction between hosts and symbionts: ideas and evidence. J Genet 96:483–489. https://doi.org/10.1007/s12041-017-0792-9.
53. Kopac SM, Klassen JL. 2016. Can they make it on their own? Hosts, microbes, and the holobiont niche. Front Microbiol 7:1647. https://doi.org/10.3389/fmicb.2016.01647.
54. Wagner GP, Altenberg L. 1996. Complex adaptations and the evolution of evolvability. Evolution 50:967–976. https://doi.org/10.1111/j.1558-5646.1996.tb02339.x.
55. Baquero F, Tedim AP, Coque TM. 2013. Antibiotic resistance shaping multi-level population biology of bacteria. Front Microbiol 4:15. https://doi.org/10.3389/fmicb.2013.00015.
56. Bershtein S, Serohijos AW, Shakhnovich EI. 2017. Bridging the physical scales in evolutionary biology: from protein sequence space to fitness of organisms and populations. Curr Opin Struct Biol 42:31–40. https://doi.org/10.1016/j.sbi.2016.10.013.
57. Lopatkin AJ, Stokes JM, Zheng EJ, Yang JH, Takahashi MK, You L, Collins JJ. 2019. Bacterial metabolic state more accurately predicts antibiotic lethality than growth rate. Nat Microbiol 4:2109–2117. https://doi.org/10.1038/s41564-019-0536-0.
58. Yang JH, Wright SN, Hamblin M, McCloskey D, Alcantar MA, Schrübbers L, Lopatkin AJ, Satish S, Nili A, Palsson BO, Walker GC, Collins JJ. 2019. A white-box machine learning approach for revealing antibiotic mechanisms of action. Cell 177:1649–1661.e9. https://doi.org/10.1016/j.cell.2019.04.016.
59. Härdling R, Smith HG, Jormalainen V, Tuomi J. 2001. Resolution of evolutionary conflicts: costly behaviours enforce the evolution of cost-free competition. Evol Ecol Res 3:829–844.
60. Darwin C. 1964. On the origin of species: a facsimile of the first edition with an introduction by Ernst Mayr. Harvard University Press, Cambridge, MA.
61. Croucher NJ, Klugman KP. 2014. The emergence of bacterial "hopeful monsters." mBio 5:e01550-14. https://doi.org/10.1128/mBio.01550-14.
62. Dittrich-Reed DR, Fitzpatrick BM. 2013. Transgressive hybrids as hopeful monsters. Evol Biol 40:310–315. https://doi.org/10.1007/s11692-012-9209-0.
63. Gillespie JH. 2001. Is the population size of a species relevant to its evolution? Evolution 55:2161–2169. https://doi.org/10.1111/j.0014-3820.2001.tb00732.x.
64. Masel J. 2011. Genetic drift. Curr Biol 21:R837–R838. https://doi.org/10.1016/j.cub.2011.08.007.
65. Tyerman JG, Ponciano JM, Joyce P, Forney LJ, Harmon LJ. 2013. The evolution of antibiotic susceptibility and resistance during the formation of Escherichia coli biofilms in the absence of antibiotics. BMC Evol Biol 13:22. https://doi.org/10.1186/1471-2148-13-22.
66. Steenackers HP, Parijs I, Dubey A, Foster KR, Vanderleyden J. 2016. Experimental evolution in biofilm populations. FEMS Microbiol Rev 40:373–397. https://doi.org/10.1093/femsre/fuw002.
67. Stenström TA. 1989. Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. Appl Environ Microbiol 55:142–147. https://doi.org/10.1128/AEM.55.1.142-147.1989.
68. Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, Sabanay I, Mahalu D, Safran S, Bershadsky A, Addadi L, Geiger B. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat Cell Biol 3:466–472. https://doi.org/10.1038/35074532.
69. Novais Â, Comas I, Baquero F, Cantón R, Coque TM, Moya A, González-Candelas FG, Galán JC. 2010. Evolutionary trajectories of beta-lactamase CTX-M-1 cluster enzymes: predicting antibiotic resistance. PLoS Pathog 6:e1000735. https://doi.org/10.1371/journal.ppat.1000735.
70. Weinreich DM, Chao L. 2005. Rapid evolutionary escape by large populations from local fitness peaks is likely in nature. Evolution 59:1175–1182. https://doi.org/10.1111/j.0014-3820.2005.tb01769.x.
71. Gillespie JH. 2000. The neutral theory in an infinite population. Gene 261:11–18. https://doi.org/10.1016/S0378-1119(00)00485-6.
72. Koonin EV, Wolf YI. 2012. Evolution of microbes and viruses: a paradigm shift in evolutionary biology? Front Cell Infect Microbiol 2:119. https://doi.org/10.3389/fcimb.2012.00119.
73. Hanson-Smith V, Johnson A. 2016. PhyloBot: a web portal for automated phylogenetics, ancestral sequence reconstruction, and exploration of mutational trajectories. PLoS Comput Biol 12:e1004976. https://doi.org/10.1371/journal.pcbi.1004976.
74. Gonzalez-Alba JM, Baquero F, Cantón R, Galán JC. 2019. Stratified reconstruction of ancestral Escherichia coli diversification. BMC Genomics 20:936. https://doi.org/10.1186/s12864-019-6346-1.
75. Turrientes MC, González-Alba JM, Del Campo R, Baquero MR, Cantón R, Baquero F, Galán JC. 2014. Recombination blurs phylogenetic groups routine assignment in Escherichia coli: setting the record straight. PLoS One 9:e105395. https://doi.org/10.1371/journal.pone.0105395.
76. Soucy SM, Huang J, Gogarten JP. 2015. Horizontal gene transfer: building the web of life. Nat Rev Genet 16:472–482. https://doi.org/10.1038/nrg3962.
77. Bapteste E, Bouchard F, Burian RM. 2012. Philosophy and evolution: minding the gap between evolutionary patterns and tree-like patterns. Methods Mol Biol 856:81–110. https://doi.org/10.1007/978-1-61779-585-5_4.
78. Corel E, Lopez P, Méheust R, Bapteste E. 2016. Network-thinking: graphs to analyze microbial complexity and evolution. Trends Microbiol 24:224–237. https://doi.org/10.1016/j.tim.2015.12.003.
79. Lerat E, Daubin V, Ochman H, Moran NA. 2005. Evolutionary origins of genomic repertoires in bacteria. PLoS Biol 3:e130. https://doi.org/10.1371/journal.pbio.0030130.
80. Lanza VF, Baquero F, De La CF, Coque TM. 2017. AcCNET (Accessory Genome Constellation Network): comparative genomics software for accessory genome analysis using bipartite networks. Bioinformatics 33:283–285. https://doi.org/10.1093/bioinformatics/btw601.
81. McShea D, Brandon R. 2010. Biology's first law: the tendency for diversity and complexity to increase in evolutionary systems. University of Chicago Press, Chicago, IL.
82. Baquero F. 2011. The 2010 Garrod Lecture: the dimensions of evolution in antibiotic resistance: *ex unibus plurum* et *ex pluribus unum*. J Antimicrob Chemother 66:1659–1672. https://doi.org/10.1093/jac/dkr214.
83. Kinnersley MA, Holben WE, Rosenzweig F. 2009. *E unibus plurum*: genomic analysis of an experimentally evolved polymorphism in

Escherichia coli. PLoS Genet 5:e1000713. https://doi.org/10.1371/journal.pgen.1000713.
84. Novak S, Chatterjee K, Nowak MA. 2013. Density games. J Theor Biol 334:26–34. https://doi.org/10.1016/j.jtbi.2013.05.029.
85. Reding-Roman C, Hewlett M, Duxbury S, Gori F, Gudelj I, Beardmore R. 2017. The unconstrained evolution of fast and efficient antibiotic-resistant bacterial genomes. Nat Ecol Evol 1:50. https://doi.org/10.1038/s41559-016-0050.
86. Yu Y, Xiao G, Zhou J, Wang Y, Wang Z, Kurths J, Joachim Schellnhuber H. 2016. System crash as dynamics of complex networks. Proc Natl Acad Sci U S A 113:11726–11731. https://doi.org/10.1073/pnas.1612094113.
87. Bódi Z, Farkas Z, Nevozhay D, Kalapis D, Lázár V, Csörgő B, Nyerges Á, Szamecz B, Fekete G, Papp B, Araújo H, Oliveira JL, Moura G, Santos MAS, Székely T, Balázsi G, Pál C. 2017. Phenotypic heterogeneity promotes adaptive evolution. PLoS Biol 15:e1002607. https://doi.org/10.1371/journal.pbio.1002607.
88. Sánchez-Romero MA, Casadesús J. 2014. Contribution of phenotypic heterogeneity to adaptive antibiotic resistance. Proc Natl Acad Sci U S A 111:355–360. https://doi.org/10.1073/pnas.1316084111.
89. Veening JW, Smits WK, Kuipers OP. 2008. Bistability, epigenetics, and bet-hedging in bacteria. Annu Rev Microbiol 62:193–210. https://doi.org/10.1146/annurev.micro.62.081307.163002.
90. Rubin IN, Doebeli M. 2017. Rethinking the evolution of specialization: a model for the evolution of phenotypic heterogeneity. J Theor Biol 435:248–264. https://doi.org/10.1016/j.jtbi.2017.09.020.
91. Ho WC, Zhang J. 2018. Evolutionary adaptations to new environments generally reverse plastic phenotypic changes. Nat Commun 9:833. https://doi.org/10.1038/s41467-018-03360-3.
92. De Jong IG, Haccou P, Kuipers OP. 2011. Bet hedging or not? A guide to proper classification of microbial survival strategies. Bioessays 33:215–223. https://doi.org/10.1002/bies.201000127.
93. Nicoloff H, Hjort K, Levin BR, Andersson DI. 2019. The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification. Nat Microbiol 4:504–514. https://doi.org/10.1038/s41564-018-0342-0.
94. Saxer G, Krepps MD, Merkley ED, Ansong C, Deatherage Kaiser BL, Valovska MT, Ristic N, Yeh PT, Prakash VP, Leiser OP, Nakhleh L, Gibbons HS, Kreuzer HW, Shamoo Y. 2014. Mutations in global regulators lead to metabolic selection during adaptation to complex environments. PLoS Genet 10:e1004872. https://doi.org/10.1371/journal.pgen.1004872.
95. Johnson PJT, Levin BR. 2013. Pharmacodynamics, population dynamics, and the evolution of persistence in *Staphylococcus aureus*. PLoS Genet 9:e1003123. https://doi.org/10.1371/journal.pgen.1003123.
96. Day T. 2016. Interpreting phenotypic antibiotic tolerance and persister cells as evolution via epigenetic inheritance. Mol Ecol 25:1869–1882. https://doi.org/10.1111/mec.13603.
97. Drummond AD, Wilke CO. 2009. The evolutionary consequences of erroneous protein synthesis. Nat Rev Genet 10:715–724. https://doi.org/10.1038/nrg2662.
98. Ribas de Pouplana L, Santos MAS, Zhu JH, Farabaugh PJ, Javid B. 2014. Protein mistranslation: friend or foe? Trends Biochem Sci 39:355–362. https://doi.org/10.1016/j.tibs.2014.06.002.
99. Javid B, Sorrentino F, Toosky M, Zheng W, Pinkham JT, Jain N, Pan M, Deighan P, Rubin EJ. 2014. Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. Proc Natl Acad Sci U S A 111:1132–1137. https://doi.org/10.1073/pnas.1317580111.
100. Saito N, Ishihara S, Kaneko K. 2013. Baldwin effect under multipeaked fitness landscapes: phenotypic fluctuation accelerates evolutionary rate. Phys Rev E Stat Nonlin Soft Matter Phys 87:e052701. https://doi.org/10.1103/PhysRevE.87.052701.
101. Martinez JL, Baquero F. 2000. Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother 44:1771–1777. https://doi.org/10.1128/aac.44.7.1771-1777.2000.
102. Gordo I. 2019. Evolutionary change in the human gut microbiome: from a static to a dynamic view. PLoS Biol 17:e3000126. https://doi.org/10.1371/journal.pbio.3000126.
103. Yang L, Jelsbak L, Marvig RL, Damkiaer S, Workman CT, Rau MH, Hansen SK, Folkesson A, Johansen HK, Ciofu O, Hoiby N, Sommer MOA, Molin S. 2011. Evolutionary dynamics of bacteria in a human host environment. Proc Natl Acad Sci U S A 108:7481–7486. https://doi.org/10.1073/pnas.1018249108.
104. Moxon R, Bayliss C, Hood D. 2006. Bacterial contingency loci: the role of simple sequence DNA repeats in bacterial adaptation. Annu Rev Genet 40:307–333. https://doi.org/10.1146/annurev.genet.40.110405.090442.
105. De Ste Croix M, Holmes J, Wanford JJ, Moxon ER, Oggioni MR, Bayliss CD. 2020. Selective and non-selective bottlenecks as drivers of the evolution of hypermutable bacterial loci. Mol Microbiol 113:672–681. https://doi.org/10.1111/mmi.14453.
106. Foster PL, Hanson AJ, Lee H, Popodi EM, Tang H. 2013. On the mutational topology of the bacterial genome. G3 (Bethesda) 3:399–407. https://doi.org/10.1534/g3.112.005355.
107. Baquero M-R, Nilsson AI, del Carmen Turrientes M, Sandvang D, Galán JC, Martínez JL, Frimodt-Møller N, Baquero F, Andersson DI, 2004. Polymorphic mutation frequencies in *Escherichia coli*: emergence of weak mutators in clinical isolates. J Bacteriol 186:5538–5542. https://doi.org/10.1128/JB.186.16.5538-5542.2004.
108. Miller JH. 1996. Spontaneous mutators in bacteria: insights into pathways of mutagenesis and repair. Annu Rev Microbiol 50:625–643. https://doi.org/10.1146/annurev.micro.50.1.625.
109. Couce A, Guelfo JR, Blázquez J. 2013. Mutational spectrum drives the rise of mutator bacteria. PLoS Genet 9:e1003167. https://doi.org/10.1371/journal.pgen.1003167.
110. Hammerstrom TG, Beabout K, Clements TP, Saxer G, Shamoo Y. 2015. *Acinetobacter baumannii* repeatedly evolves a hypermutator phenotype in response to tigecycline that effectively surveys evolutionary trajectories to resistance. PLoS One 10:e0140489. https://doi.org/10.1371/journal.pone.0140489.
111. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. 2000. High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. Science 288:1251–1253. https://doi.org/10.1126/science.288.5469.1251.
112. Baquero MR, Galán JC, Turrientes MDC, Cantón R, Coque TM, Martínez JL, Baquero F. 2005. Increased mutation frequencies in Escherichia coli isolates harboring extended-spectrum β-lactamases. Antimicrob Agents Chemother 49:4754–4756. https://doi.org/10.1128/AAC.49.11.4754-4756.2005.
113. Raynes Y, Sniegowski PD. 2014. Experimental evolution and the dynamics of genomic mutation rate modifiers. Heredity (Edinb) 113:375–380. https://doi.org/10.1038/hdy.2014.49.
114. Wilke CO, Wang JL, Ofria C, Lenski RE, Adami C. 2001. Evolution of digital organisms at high mutation rates leads to survival of the flattest. Nature 412:331–333. https://doi.org/10.1038/35085569.
115. Tejero H, Marín A, Montero F. 2011. The relationship between the error catastrophe, survival of the flattest, and natural selection. BMC Evol Biol 11:2. https://doi.org/10.1186/1471-2148-11-2.
116. Schneider D, Lenski RE. 2004. Dynamics of insertion sequence elements during experimental evolution of bacteria. Res Microbiol 155:319–327. https://doi.org/10.1016/j.resmic.2003.12.008.
117. Siguier P, Gourbeyre E, Chandler M. 2014. Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiol Rev 38:865–891. https://doi.org/10.1111/1574-6976.12067.
118. Fehér T, Bogos B, Méhi O, Fekete G, Csörg B, Kovács K, Pósfai G, Papp B, Hurst LD, Pál C. 2012. Competition between transposable elements and mutator genes in bacteria. Mol Biol Evol 29:3153–3159. https://doi.org/10.1093/molbev/mss122.
119. Pomiankowski A. 1999. Intragenomic conflict, p 121–152. *In* Keller J (ed), Levels of selection in evolution. Princeton University Press, Princeton, NJ.
120. Martinez JL, Cercenado E, Rodriguez-Creixems M, Vincente-Perez MF, Delgado-Iribarren A, Baquero F. 1987. Resistance to beta-lactam/clavulanate. Lancet ii:1473. https://doi.org/10.1016/S0140-6736(87)91180-9.
121. Sandegren L, Andersson DI. 2009. Bacterial gene amplification: implications for the evolution of antibiotic resistance. Nat Rev Microbiol 7:578–588. https://doi.org/10.1038/nrmicro2174.
122. Dhar R, Bergmiller T, Wagner A. 2014. Increased gene dosage plays a predominant role in the initial stages of evolution of duplicate TEM-1 beta lactamase genes. Evolution 68:1775–1791. https://doi.org/10.1111/evo.12373.
123. Maisnier-Patin S, Roth JR. 2015. The origin of mutants under selection: how natural selection mimics mutagenesis (adaptive mutation). Cold Spring Harb Perspect Biol 7:a018176. https://doi.org/10.1101/cshperspect.a018176.
124. Andersson DI, Nicoloff H, Hjort K. 2019. Mechanisms and clinical relevance of bacterial heteroresistance. Nat Rev Microbiol 17:479–496. https://doi.org/10.1038/s41579-019-0218-1.
125. Millan AS, Escudero JA, Gifford DR, Mazel D, MacLean RC. 2017. Multicopy plasmids potentiate the evolution of antibiotic resistance in bacteria. Nat Ecol Evol 1:10. https://doi.org/10.1038/s41559-016-0010.
126. Rodriguez-Beltran J, Hernandez-Beltran JCR, Delafuente J, Escudero JA, Fuentes-Hernandez A, MacLean RC, Peña-Miller R, San Millan A. 2018. Multicopy plasmids allow bacteria to escape from fitness trade-offs

during evolutionary innovation. Nat Ecol Evol 2:873–881. https://doi.org/10.1038/s41559-018-0529-z.
127. Oliveira PH, Touchon M, Cury J, Rocha EPC. 2017. The chromosomal organization of horizontal gene transfer in bacteria. Nat Commun 8:841. https://doi.org/10.1038/s41467-017-00808-w.
128. Puigbò P, Lobkovsky AE, Kristensen DM, Wolf YI, Koonin EV. 2014. Genomes in turmoil: quantification of genome dynamics in prokaryote supergenomes. BMC Med 12:66.
129. Sanz-García F, Hernando-Amado S, Martínez JL. 2018. Mutation-driven evolution of pseudomonas aeruginosa in the presence of either ceftazidime or ceftazidime-avibactam. Antimicrob Agents Chemother 62: e01379-18. https://doi.org/10.1128/AAC.01379-18.
130. Hernando-Amado S, Sanz-García F, Martínez JL. 2020. Rapid and robust evolution of collateral sensitivity in Pseudomonas aeruginosa antibiotic-resistant mutants. Sci Adv 6:eaba5493. https://doi.org/10.1126/sciadv.aba5493.
131. Carja O, Liberman U, Feldman MW. 2013. Evolution with stochastic fitnesses: a role for recombination. Theor Popul Biol 86:29–42. https://doi.org/10.1016/j.tpb.2013.02.005.
132. Price VJ, McBride SW, Hullahalli K, Chatterjee A, Duerkop BA, Palmer KL. 2019. *Enterococcus faecalis* CRISPR-cas is a robust barrier to conjugative antibiotic resistance dissemination in the murine intestine. mSphere 4: e00464-19. https://doi.org/10.1128/mSphere.00464-19.
133. Tang Y, Fu P, Zhou Y, Xie Y, Jin J, Wang B, Yu L, Huang Y, Li G, Li M, Liang W, Ou HY, Jiang X. 2020. Absence of the type I-E CRISPR-Cas system in *Klebsiella pneumoniae* clonal complex 258 is associated with dissemination of IncF epidemic resistance plasmids in this clonal complex. J Antimicrob Chemother 75:890–895. https://doi.org/10.1093/jac/dkz538.
134. Foster PL. 2007. Stress-induced mutagenesis in bacteria. Crit Rev Biochem Mol Biol 42:373–397. https://doi.org/10.1080/10409230701648494.
135. Balbontín R, Frazão N, Gordo I. 2021. DNA breaks-mediated cost reveals RNase HI as a new target for selectively eliminating antibiotic resistance. bioRxiv 2021:756767.
136. Baharoglu Z, Mazel D. 2014. SOS, the formidable strategy of bacteria against aggressions. FEMS Microbiol Rev 38:1126–1145. https://doi.org/10.1111/1574-6976.12077.
137. Baquero F, Levin BR. 2021. Proximate and ultimate causes of the bactericidal action of antibiotics. Nat Rev Microbiol 19:123–132. https://doi.org/10.1038/s41579-020-00443-1.
138. Gutierrez A, Laureti L, Crussard S, Abida H, Rodríguez-Rojas A, Blázquez J, Baharoglu Z, Mazel D, Darfeuille F, Vogel J, Matic I. 2013. β-Lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. Nat Commun 4:1610. https://doi.org/10.1038/ncomms2607.
139. Anciaux Y, Chevin LM, Ronce O, Martin G. 2018. Evolutionary rescue over a fitness landscape. Genetics 209:265–279. https://doi.org/10.1534/genetics.118.300908.
140. Meehan MT, Cope RC, McBryde ES. 2020. On the probability of strain invasion in endemic settings: accounting for individual heterogeneity and control in multi-strain dynamics. J Theor Biol 487:110109. https://doi.org/10.1016/j.jtbi.2019.110109.
141. Imsande J. 1978. Genetic regulation of penicillinase synthesis in gram-positive bacteria. Microbiol Rev 42:67–83. https://doi.org/10.1128/MR.42.1.67-83.1978.
142. Weisblum B, Siddhikol C, Lai CJ, Demohn V. 1971. Erythromycin-inducible resistance in *Staphylococcus aureus*: requirements for induction. J Bacteriol 106:835–847. https://doi.org/10.1128/JB.106.3.835-847.1971.
143. Reig M, Fernandez MC, Ballesta JPG, Baquero F. 1992. Inducible expression of ribosomal clindamycin resistance in *Bacteroides vulgatus*. Antimicrob Agents Chemother 36:639–642. https://doi.org/10.1128/aac.36.3.639.
144. Linares JF, Gustafsson I, Baquero F, Martinez JL. 2006. Antibiotics as intermicrobial signaling agents instead of weapons. Proc Natl Acad Sci U S A 103:19484–19489. https://doi.org/10.1073/pnas.0608949103.
145. Vadlamani G, Thomas MD, Patel TR, Donald LJ, Reeve TM, Stetefeld J, Standing KG, Vocadlo DJ, Mark BL. 2015. The β-lactamase gene regulator AmpR is a tetramer that recognizes and binds the D-Ala-D-Ala motif of its repressor UDP-N-acetylmuramic acid (MurNAc)-pentapeptide. J Biol Chem 290:2630–2643. https://doi.org/10.1074/jbc.M114.618199.
146. Courvalin P. 2006. Vancomycin resistance in gram-positive cocci. Clin Infect Dis 42:S24–S34.
147. Mitrophanov AY, Groisman EA. 2008. Signal integration in bacterial two-component regulatory systems. Genes Dev 22:2601–2611. https://doi.org/10.1101/gad.1700308.
148. Blanco P, Hernando-Amado S, Reales-Calderon J, Corona F, Lira F, Alcalde-Rico M, Bernardini A, Sanchez M, Martinez J. 2016. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms 4:14. https://doi.org/10.3390/microorganisms4010014.
149. Rosenberg EY, Bertenthal D, Nilles ML, Bertrand KP, Nikaido H. 2003. Bile salts and fatty acids induce the expression of *Escherichia coli* AcrAB multidrug efflux pump through their interaction with Rob regulatory protein. Mol Microbiol 48:1609–1619. https://doi.org/10.1046/j.1365-2958.2003.03531.x.
150. García-León G, Hernández A, Hernando-Amado S, Alavi P, Berg G, Martínez JL. 2014. A function of SmeDEF, the major quinolone resistance determinant of *Stenotrophomonas maltophilia*, is the colonization of plant roots. Appl Environ Microbiol 80:4559–4565. https://doi.org/10.1128/AEM.01058-14.
151. Callahan HS, Maughan H, Steiner UK. 2008. Phenotypic plasticity, costs of phenotypes, and costs of plasticity: toward an integrative view. Ann N Y Acad Sci 1133:44–66. https://doi.org/10.1196/annals.1438.008.
152. McNally A, Oren Y, Kelly D, Pascoe B, Dunn S, Sreecharan T, Vehkala M, Välimäki N, Prentice MB, Ashour A, Avram O, Pupko T, Dobrindt U, Literak I, Guenther S, Schaufler K, Wieler LH, Zhiyong Z, Sheppard SK, McInerney JO, Corander J. 2016. Combined analysis of variation in core, accessory and regulatory genome regions provides a super-resolution view into the evolution of bacterial populations. PLoS Genet 12: e1006280. https://doi.org/10.1371/journal.pgen.1006280.
153. Brauner A, Fridman O, Gefen O, Balaban NQ. 2016. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Microbiol 14:320–330. https://doi.org/10.1038/nrmicro.2016.34.
154. Germain E, Roghanian M, Gerdes K, Maisonneuve E. 2015. Stochastic induction of persister cells by HipA through (p)ppGpp-mediated activation of mRNA endonucleases. Proc Natl Acad Sci U S A 112:5171–5176. https://doi.org/10.1073/pnas.1423536112.
155. Levin BR, Rozen DE. 2006. Non-inherited antibiotic resistance. Nat Rev Microbiol 4:556–562. https://doi.org/10.1038/nrmicro1445.
156. Liu J, Gefen O, Ronin I, Bar-Meir M, Balaban NQ. 2020. Effect of tolerance on the evolution of antibiotic resistance under drug combinations. Science 367:200–204. https://doi.org/10.1126/science.aay3041.
157. Bakkeren E, Huisman JS, Fattinger SA, Hausmann A, Furter M, Egli A, Slack E, Sellin ME, Bonhoeffer S, Regoes RR, Diard M, Hardt WD. 2019. *Salmonella* persisters promote the spread of antibiotic resistance plasmids in the gut. Nature 573:276–280. https://doi.org/10.1038/s41586-019-1521-8.
158. Levin-Reisman I, Brauner A, Ronin I, Balaban NQ. 2019. Epistasis between antibiotic tolerance, persistence, and resistance mutations. Proc Natl Acad Sci U S A 116:14734–14739. https://doi.org/10.1073/pnas.1906169116.
159. Mathieu A, Fleurier S, Frénoy A, Dairou J, Bredeche MF, Sanchez-Vizuete P, Song X, Matic I. 2016. Discovery and function of a general core hormetic stress response in *E. coli* induced by sublethal concentrations of antibiotics. Cell Rep 17:46–57. https://doi.org/10.1016/j.celrep.2016.09.001.
160. Rodríguez-Rojas A, Makarova O, Rolff J. 2014. Antimicrobials, stress and mutagenesis. PLoS Pathog 10:e1004445. https://doi.org/10.1371/journal.ppat.1004445.
161. Windels EM, Van Den Bergh B, Michiels J. 2020. Bacteria under antibiotic attack: different strategies for evolutionary adaptation. PLoS Pathog 16: e1008431. https://doi.org/10.1371/journal.ppat.1008431.
162. Alexander HK, MacLean RC. 2020. Stochastic bacterial population dynamics restrict the establishment of antibiotic resistance from single cells. Proc Natl Acad Sci U S A 117:19455–19464. https://doi.org/10.1073/pnas.1919672117.
163. Wistrand-Yuen E, Knopp M, Hjort K, Koskiniemi S, Berg OG, Andersson DI. 2018. Evolution of high-level resistance during low-level antibiotic exposure. Nat Commun 9:1599. https://doi.org/10.1038/s41467-018-04059-1.
164. Baquero F, Negri MC. 1997. Selective compartments for resistant microorganisms in antibiotic gradients. Bioessays 19:731–736. https://doi.org/10.1002/bies.950190814.
165. Pfeifer E, Moura de Sousa JA, Touchon M, Rocha EPC. 2021. Bacteria have numerous distinctive groups of phage-plasmids with conserved phage and variable plasmid gene repertoires. Nucleic Acids Res 49:2655–2673. https://doi.org/10.1093/nar/gkab064.
166. Bernier SP, Surette MG. 2013. Concentration-dependent activity of antibiotics in natural environments. Front Microbiol 4:20. https://doi.org/10.3389/fmicb.2013.00020.

167. Negri MC, Lipsitch M, Blázquez J, Levin BR, Baquero F. 2000. Concentration-dependent selection of small phenotypic differences in TEM beta-lactamase-mediated antibiotic resistance. Antimicrob Agents Chemother 44:2485–2491. https://doi.org/10.1128/aac.44.9.2485-2491.2000.
168. Drlica K, Zhao X. 2007. Mutant selection window hypothesis updated. Clin Infect Dis 44:681–688. https://doi.org/10.1086/511642.
169. Baquero F, Coque TM. 2014. Widening the spaces of selection: evolution along sublethal antimicrobial gradients. mBio 5:e02270-14. https://doi.org/10.1128/mBio.02270-14.
170. Hermsen R, Deris JB, Hwa T. 2012. On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient. Proc Natl Acad Sci U S A 109:10775–10780. https://doi.org/10.1073/pnas.1117716109.
171. Greulich P, Waclaw B, Allen RJ. 2012. Mutational pathway determines whether drug gradients accelerate evolution of drug-resistant cells. Phys Rev Lett 109:e088101. https://doi.org/10.1103/PhysRevLett.109.088101.
172. Greenfield BK, Shaked S, Marrs CF, Nelson P, Raxter I, Xi C, McKone TE, Jolliet O. 2017. Modeling the emergence of antibiotic resistance in the environment: an analytical solution for the minimum selection concentration. Antimicrob Agents Chemother 62:e01686-17. https://doi.org/10.1128/AAC.01686-17.
173. Morrissey MB. 2014. Selection and evolution of causally covarying traits. Evolution 68:1748–1761. https://doi.org/10.1111/evo.12385.
174. Landecker H. 2019. Antimicrobials before antibiotics: war, peace, and disinfectants. Palgrave Commun 5:45. https://doi.org/10.1057/s41599-019-0251-8.
175. Podolsky SH. 2018. The evolving response to antibiotic resistance (1945–2018). Palgrave Commun 4:124. https://doi.org/10.1057/s41599-018-0181-x.
176. Lemire JA, Harrison JJ, Turner RJ. 2013. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol 11:371–384. https://doi.org/10.1038/nrmicro3028.
177. Rebeniak M, Wojciechowska-Mazurek M, Mania M, Szynal T, Strzelecka A, Starska K. 2014. Exposure to lead and cadmium released from ceramics and glassware intended to come into contact with food. Rocz Panstw Zakl Hig 65:301–309.
178. Greenwood D. 2010. Historical introduction, p 3–10. *In* Finch R, Greenwood D, Norrby R, Whitley R (ed), Antimicrobial chemotherapy. Saunders Elsevier, Edinburgh, United Kingdom.
179. Aminov RI. 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol 1:134. https://doi.org/10.3389/fmicb.2010.00134.
180. Wright S. 1932. The roles of mutation, inbreeding, cross-breeding and selection in evolution, p 356–366. *In* Jones DF (ed), Proceedings of the 6th International Congress of Genetics. The Collegiate Press, Menasha, WI.
181. Yu G, Baeder DY, Regoes RR, Rolff J. 2018. Predicting drug resistance evolution: insights from antimicrobial peptides and antibiotics. Proc R Soc B 285:20172687. https://doi.org/10.1098/rspb.2017.2687.
182. Zagorski M, Burda Z, Waclaw B. 2016. Beyond the Hypercube: evolutionary accessibility of fitness landscapes with realistic mutational networks. PLoS Comput Biol 12:e1005218. https://doi.org/10.1371/journal.pcbi.1005218.
183. Ferretti L, Weinreich D, Tajima F, Achaz G. 2018. Evolutionary constraints in fitness landscapes. Heredity (Edinb) 121:466–481. https://doi.org/10.1038/s41437-018-0110-1.
184. Palmer AC, Toprak E, Baym M, Kim S, Veres A, Bershtein S, Kishony R. 2015. Delayed commitment to evolutionary fate in antibiotic resistance fitness landscapes. Nat Commun 6:7385. https://doi.org/10.1038/ncomms8385.
185. Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I, Whitlock MC. 2012. Experimental evolution. Trends Ecol Evol 27:547–560. https://doi.org/10.1016/j.tree.2012.06.001.
186. Steinberg B, Ostermeier M. 2016. Shifting fitness and epistatic landscapes reflect trade-offs along an evolutionary pathway. J Mol Biol 428:2730–2743. https://doi.org/10.1016/j.jmb.2016.04.033.
187. Ohta T. 1972. Evolutionary rate of cistrons and DNA divergence. J Mol Evol 1:150–157. https://doi.org/10.1007/BF01659161.
188. Sella G, Hirsh AE. 2005. The application of statistical physics to evolutionary biology. Proc Natl Acad Sci U S A 102:9541–9546. https://doi.org/10.1073/pnas.0501865102.
189. Weissman DB, Desai MM, Fisher DS, Feldman MW. 2009. The rate at which asexual populations cross fitness valleys. Theor Popul Biol 75:286–300. https://doi.org/10.1016/j.tpb.2009.02.006.
190. Jain K. 2007. Evolutionary dynamics of the most populated genotype on rugged fitness landscapes. Phys Rev E Stat Nonlin Soft Matter Phys 76:e031922. https://doi.org/10.1103/PhysRevE.76.031922.
191. Altland A, Fischer A, Krug J, Szendro IG. 2011. Rare events in population genetics: stochastic tunneling in a two-locus model with recombination. Phys Rev Lett 106:e088101.
192. Ochs IE, Desai MM. 2015. The competition between simple and complex evolutionary trajectories in asexual populations. BMC Evol Biol 15:55. https://doi.org/10.1186/s12862-015-0334-0.
193. Gottesman O, Andrejevic J, Rycroft CH, Rubinstein SM. 2018. A state variable for crumpled thin sheets. Commun Phys 1:70. https://doi.org/10.1038/s42005-018-0072-x.
194. Cambou AD, Menon N. 2011. Three-dimensional structure of a sheet crumpled into a ball. Proc Natl Acad Sci U S A 108:14741–14745. https://doi.org/10.1073/pnas.1019192108.
195. Baquero F, Martínez JL, Cantón R. 2008. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol 19:260–265. https://doi.org/10.1016/j.copbio.2008.05.006.
196. Remold SK, Lenski RE. 2001. Contribution of individual random mutations to genotype-by-environment interactions in *Escherichia coli*. Proc Natl Acad Sci U S A 98:11388–11393. https://doi.org/10.1073/pnas.201140198.
197. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Bürgmann H, Sørum H, Norström M, Pons MN, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL. 2015. Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13:310–317. https://doi.org/10.1038/nrmicro3439.
198. Larsson DGJ, Andremont A, Bengtsson-Palme J, Brandt KK, de Roda Husman AM, Fagerstedt P, Fick J, Flach CF, Gaze WH, Kuroda M, Kvint K, Laxminarayan R, Manaia CM, Nielsen KM, Plant L, Ploy MC, Segovia C, Simonet P, Smalla K, Snape J, Topp E, van Hengel AJ, Verner-Jeffreys DW, Virta MPJ, Wellington EM, Wernersson AS. 2018. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. Environ Int 117:132–138. https://doi.org/10.1016/j.envint.2018.04.041.
199. Richardson EJ, Bacigalupe R, Harrison EM, Weinert LA, Lycett S, Vrieling M, Robb K, Hoskisson PA, Holden MTG, Feil EJ, Paterson GK, Tong SYC, Shittu A, van Wamel W, Aanensen DM, Parkhill J, Peacock SJ, Corander J, Holmes M, Fitzgerald JR. 2018. Gene exchange drives the ecological success of a multi-host bacterial pathogen. Nat Ecol Evol 2:1468–1478. https://doi.org/10.1038/s41559-018-0617-0.
200. Hu Y, Yang X, Li J, Lv N, Liu F, Wu J, Lin IYC, Wu N, Weimer BC, Gao GF, Liu Y, Zhu B. 2016. The bacterial mobile resistome transfer network connecting the animal and human microbiomes. Appl Environ Microbiol 82:6672–6681. https://doi.org/10.1128/AEM.01802-16.
201. Baquero F, Coque TM, Martínez JL, Aracil-Gisbert S, Lanza VF. 2019. Gene transmission in the One Health microbiosphere and the channels of antimicrobial resistance. Front Microbiol 10:2892. https://doi.org/10.3389/fmicb.2019.02892.
202. Kim W, Racimo F, Schluter J, Levy SB, Foster KR. 2014. Importance of positioning for microbial evolution. Proc Natl Acad Sci U S A 111:E1639–-E1647. https://doi.org/10.1073/pnas.1323632111.
203. Rainey PB, Travisano M. 1998. Adaptive radiation in a heterogeneous environment. Nature 394:69–72. https://doi.org/10.1038/27900.
204. Kim W, Levy SB, Foster KR. 2016. Rapid radiation in bacteria leads to a division of labour. Nat Commun 7:10508. https://doi.org/10.1038/ncomms10508.
205. Marbouty M, Koszul R. 2015. Metagenome analysis exploiting high-throughput chromosome conformation capture (3C) data. Trends Genet 31:673–682. https://doi.org/10.1016/j.tig.2015.10.003.
206. Flot JF, Marie-Nelly H, Koszul R. 2015. Contact genomics: scaffolding and phasing (meta)genomes using chromosome 3D physical signatures. FEBS Lett 589:2966–2974. https://doi.org/10.1016/j.febslet.2015.04.034.
207. Zhang Z, Geng J, Tang X, Fan H, Xu J, Wen X, Ma Z, Shi P. 2014. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. ISME J 8:881–893. https://doi.org/10.1038/ismej.2013.185.
208. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, Amieva MR, Huang KC, Sonnenburg JL. 2015. Quantitative imaging of gut microbiota spatial organization. Cell Host Microb 18:478–488. https://doi.org/10.1016/j.chom.2015.09.002.
209. Porse A, Schou TS, Munck C, Ellabaan MMH, Sommer MOA. 2018. Biochemical mechanisms determine the functional compatibility of heterologous genes. Nat Commun 9:522. https://doi.org/10.1038/s41467-018-02944-3.

210. Ford PJ, Avison MB. 2004. Evolutionary mapping of the SHV β-lactamase and evidence for two separate IS26-dependent *bla*SHV mobilization events from the *Klebsiella pneumoniae* chromosome. J Antimicrob Chemother 54:69–75. https://doi.org/10.1093/jac/dkh251.
211. Ivankov DN. 2017. Exact correspondence between walk in nucleotide and protein sequence spaces. PLoS One 12:e0182525. https://doi.org/10.1371/journal.pone.0182525.
212. Huang W, Le QQ, LaRocco M, Palzkill T. 1994. Effect of threonine-to-methionine substitution at position 265 on structure and function of TEM-1 β-lactamase. Antimicrob Agents Chemother 38:2266–2269. https://doi.org/10.1128/aac.38.10.2266.
213. Bratulic S, Gerber F, Wagner A. 2015. Mistranslation drives the evolution of robustness in TEM-1 β-lactamase. Proc Natl Acad Sci U S A 112:12758–12763. https://doi.org/10.1073/pnas.1510071112.
214. Hammerling MJ, Gollihar J, Mortensen C, Alnahhas RN, Ellington AD, Barrick JE. 2016. Expanded genetic codes create new mutational routes to rifampicin resistance in *Escherichia coli*. Mol Biol Evol 33:2054–2063. https://doi.org/10.1093/molbev/msw094.
215. Zhu W, Freeland S. 2006. The standard genetic code enhances adaptive evolution of proteins. J Theor Biol 239:63–70. https://doi.org/10.1016/j.jtbi.2005.07.012.
216. Massey SE. 2008. A neutral origin for error minimization in the genetic code. J Mol Evol 67:510–516. https://doi.org/10.1007/s00239-008-9167-4.
217. Podgornaia AI, Laub MT. 2015. Pervasive degeneracy and epistasis in a protein-protein interface. Science 347:673–677. https://doi.org/10.1126/science.1257360.
218. Pande S, Merker H, Bohl K, Reichelt M, Schuster S, De Figueiredo LF, Kaleta C, Kost C. 2014. Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. ISME J 8:953–962. https://doi.org/10.1038/ismej.2013.211.
219. Gu Z, Steinmetz LM, Gu X, Scharfe C, Davis RW, Li WH. 2003. Role of duplicate genes in genetic robustness against null mutations. Nature 421:63–66. https://doi.org/10.1038/nature01198.
220. Pasek S, Risler JL, Brézellec P. 2006. The role of domain redundancy in genetic robustness against null mutations. J Mol Biol 362:184–191. https://doi.org/10.1016/j.jmb.2006.07.033.
221. Espinosa-Soto C. 2016. Selection for distinct gene expression properties favours the evolution of mutational robustness in gene regulatory networks. J Evol Biol 29:2321–2333. https://doi.org/10.1111/jeb.12959.
222. Freihofer P, Akbergenov R, Teo Y, Juskeviciene R, Andersson DI, Böttger EC. 2016. Nonmutational compensation of the fitness cost of antibiotic resistance in mycobacteria by overexpression of tlyA rRNA methylase. RNA 22:1836–1843. https://doi.org/10.1261/rna.057257.116.
223. Björkman J, Nagaev I, Berg OG, Hughes D, Andersson DI. 2000. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. Science 287:1479–1482. https://doi.org/10.1126/science.287.5457.1479.
224. Andersson DI. 2006. The biological cost of mutational antibiotic resistance: any practical conclusions? Curr Opin Microbiol 9:461–465. https://doi.org/10.1016/j.mib.2006.07.002.
225. Harmand N, Gallet R, Jabbour-Zahab R, Martin G, Lenormand T. 2017. Fisher's geometrical model and the mutational patterns of antibiotic resistance across dose gradients. Evolution 71:23–37. https://doi.org/10.1111/evo.13111.
226. Whitacre JM, Atamas SP. 2012. Degeneracy allows for both apparent homogeneity and diversification in populations. Biosystems 110:34–42. https://doi.org/10.1016/j.biosystems.2012.08.003.
227. Lind PA, Arvidsson L, Berg OG, Andersson DI. 2017. Variation in mutational robustness between different proteins and the predictability of fitness effects. Mol Biol Evol 34:408–418. https://doi.org/10.1093/molbev/msw239.
228. Albarracín Orio AG, Piñas GE, Cortes PR, Cian MB, Echenique J. 2011. Compensatory evolution of pbp mutations restores the fitness cost imposed by β-lactam resistance in *Streptococcus pneumoniae*. PLoS Pathog 7:e1002000. https://doi.org/10.1371/journal.ppat.1002000.
229. Angst DC, Hall AR. 2013. The cost of antibiotic resistance depends on evolutionary history in Escherichia coli. BMC Evol Biol 13:163. https://doi.org/10.1186/1471-2148-13-163.
230. Naganathan AN. 2019. Modulation of allosteric coupling by mutations: from protein dynamics and packing to altered native ensembles and function. Curr Opin Struct Biol 54:1–9. https://doi.org/10.1016/j.sbi.2018.09.004.
231. Lind PA, Tobin C, Berg OG, Kurland CG, Andersson DI. 2010. Compensatory gene amplification restores fitness after inter-species gene replacements. Mol Microbiol 75:1078–1089. https://doi.org/10.1111/j.1365-2958.2009.07030.x.
232. De Visser JAGM, Rozen DE. 2006. Clonal interference and the periodic selection of new beneficial mutations in *Escherichia coli*. Genetics 172:2093–2100. https://doi.org/10.1534/genetics.105.052373.
233. Gifford DR, Maclean RC. 2013. Evolutionary reversals of antibiotic resistance in experimental populations of *Pseudomonas Aeruginosa*. Evolution 67:2973–2981. https://doi.org/10.1111/evo.12158.
234. Krakauer DC, Plotkin JB. 2002. Redundancy, antiredundancy, and the robustness of genomes. Proc Natl Acad Sci U S A 99:1405–1409. https://doi.org/10.1073/pnas.032668599.
235. Greenspan RJ. 2001. The flexible genome. Nat Rev Genet 2:383–387. https://doi.org/10.1038/35072018.
236. Phillips PC. 2008. Epistasis—the essential role of gene interactions in the structure and evolution of genetic systems. Nat Rev Genet 9:855–867. https://doi.org/10.1038/nrg2452.
237. Oggioni MR, Coelho JR, Furi L, Knight DR, Viti C, Orefici G, Martinez JL, Freitas AT, Coque TM, Morrissey I, BIOHYPO consortium. 2015. Significant differences characterise the correlation coefficients between biocide and antibiotic susceptibility profiles in Staphylococcus aureus. Current Pharm Des 21:2054–2057. https://doi.org/10.2174/1381612821666150310103238.
238. Baquero F. 2013. Epigenetics, epistasis and epidemics. Evol Med Pub Health 2013:86–88. https://doi.org/10.1093/emph/eot009.
239. Draghi JA, Plotkin JB. 2013. Selection biases the prevalence and type of epistasis along adaptive trajectories. Evolution 67:3120–3131. https://doi.org/10.1111/evo.12192.
240. Wünsche A, Dinh DM, Satterwhite RS, Arenas CD, Stoebel DM, Cooper TF. 2017. Diminishing-returns epistasis decreases adaptability along an evolutionary trajectory. Nat Ecol Evol 1:61. https://doi.org/10.1038/s41559-016-0061.
241. Goldschmidt F, Regoes RR, Johnson DR. 2017. Successive range expansion promotes diversity and accelerates evolution in spatially structured microbial populations. ISME J 11:2112–2123. https://doi.org/10.1038/ismej.2017.76.
242. Tenaillon O, Rodríguez-Verdugo A, Gaut RL, McDonald P, Bennett AF, Long AD, Gaut BS. 2012. The molecular diversity of adaptive convergence. Science 335:457–461. https://doi.org/10.1126/science.1212986.
243. Miton CM, Tokuriki N. 2016. How mutational epistasis impairs predictability in protein evolution and design. Protein Sci 25:1260–1272. https://doi.org/10.1002/pro.2876.
244. Park S, Lehner B. 2013. Epigenetic epistatic interactions constrain the evolution of gene expression. Mol Syst Biol 9:645. https://doi.org/10.1038/msb.2013.2.
245. Otoupal PB, Erickson KE, Escalas-Bordoy A, Chatterjee A. 2017. CRISPR perturbation of gene expression alters bacterial fitness under stress and reveals underlying epistatic constraints. ACS Synth Biol 6:94–107. https://doi.org/10.1021/acssynbio.6b00050.
246. Sailer ZR, Harms MJ. 2017. High-order epistasis shapes evolutionary trajectories. PLoS Comput Biol 13:e1005541. https://doi.org/10.1371/journal.pcbi.1005541.
247. Knopp M, Andersson DI. 2018. Predictable phenotypes of antibiotic resistance mutations. mBio 9:e00770-18. https://doi.org/10.1128/mBio.00770-18.
248. Cowperthwaite MC, Economo EP, Harcombe WR, Miller EL, Meyers LA. 2008. The ascent of the abundant: how mutational networks constrain evolution. PLoS Comput Biol 4:e1000110. https://doi.org/10.1371/journal.pcbi.1000110.
249. Sauerbier J, Maurer P, Rieger M, Hakenbeck R. 2012. *Streptococcus pneumoniae* R6 interspecies transformation: genetic analysis of penicillin resistance determinants and genome-wide recombination events. Mol Microbiol 86:692–706. https://doi.org/10.1111/mmi.12009.
250. Todorova K, Maurer P, Rieger M, Becker T, Bui NK, Gray J, Vollmer W, Hakenbeck R. 2015. Transfer of penicillin resistance from *Streptococcus oralis* to *Streptococcus pneumoniae* identifies murE as resistance determinant. Mol Microbiol 97:866–880. https://doi.org/10.1111/mmi.13070.
251. Trzcinski K, Thompson CM, Gilbey AM, Dowson CG, Lipsitch M. 2006. Incremental increase in fitness cost with increased β-lactam resistance in pneumococci evaluated by competition in an infant rat nasal colonization model. J Infect Dis 193:1296–1303. https://doi.org/10.1086/501367.
252. Muñoz R, De La Campa AG. 1996. ParC subunit of DNA topoisomerase IV of *Streptococcus pneumoniae* is a primary target of fluoroquinolones and cooperates with DNA gyrase A subunit in forming resistance phenotype. Antimicrob Agents Chemother 40:2252–2257. https://doi.org/10.1128/AAC.40.10.2252.

253. Zhang G, Wang C, Sui Z, Feng J. 2015. Insights into the evolutionary trajectories of fluoroquinolone resistance in *Streptococcus pneumoniae*. J Antimicrob Chemother 70:2499–2506. https://doi.org/10.1093/jac/dkv134.
254. Prammananan T, Sander P, Springer B, Böttger EC. 1999. RecA-mediated gene conversion and aminoglycoside resistance in strains heterozygous for rRNA. Antimicrob Agents Chemother 43:447–453. https://doi.org/10.1128/AAC.43.3.447.
255. Tsakris A, Pillai SK, Gold HS, Thauvin-Eliopoulos C, Venkataraman L, Wennersten C, Moellering RC, Eliopoulos GM. 2007. Persistence of rRNA operon mutated copies and rapid re-emergence of linezolid resistance in Staphylococcus aureus. J Antimicrob Chemother 60:649–651. https://doi.org/10.1093/jac/dkm246.
256. Weinreich DM, Delaney NF, DePristo MA, Hartl DL. 2006. Darwinian evolution can follow only very few mutational paths to fitter proteins. Science 312:111–114. https://doi.org/10.1126/science.1123539.
257. Guthrie VB, Allen J, Camps M, Karchin R. 2011. Network models of TEM β-lactamase mutations coevolving under antibiotic selection show modular structure and anticipate evolutionary trajectories. PLoS Comput Biol 7:e1002184. https://doi.org/10.1371/journal.pcbi.1002184.
258. Kogenaru M, De Vos MGJ, Tans SJ. 2009. Revealing evolutionary pathways by fitness landscape reconstruction revealing evolutionary pathways by fitness landscape reconstruction. Crit Rev Biochem Mol Biol 44:169–174. https://doi.org/10.1080/10409230903039658.
259. Blazquez J, Morosini MI, Negri MC, Baquero F. 2000. Selection of naturally occurring extended-spectrum TEM β-lactamase variants by fluctuating β-lactam pressure. Antimicrob Agents Chemother 44:2182–2184. https://doi.org/10.1128/aac.44.8.2182-2184.2000.
260. Orencia MC, Yoon JS, Ness JE, Stemmer WPC, Stevens RC. 2001. Predicting the emergence of antibiotic resistance by directed evolution and structural analysis. Nat Struct Biol 8:238–242. https://doi.org/10.1038/84981.
261. Rodrigues JV, Bershtein S, Li A, Lozovsky ER, Hartl DL, Shakhnovich EI. 2016. Biophysical principles predict fitness landscapes of drug resistance. Proc Natl Acad Sci U S A 113:E1470–1478. https://doi.org/10.1073/pnas.1601441113.
262. Petrosino JF, Palzkill T. 1996. Systematic mutagenesis of the active site omega loop of TEM-I β-lactamase. J Bacteriol 178:1821–1828. https://doi.org/10.1128/JB.178.7.1821-1828.1996.
263. Santoyo G, Romero D. 2005. Gene conversion and concerted evolution in bacterial genomes. FEMS Microbiol Rev 29:169–183. https://doi.org/10.1016/j.fmrre.2004.10.004.
264. Poirel L, Naas T, Le Thomas I, Karim A, Bingen E, Nordmann P. 2001. CTX-M-type extended-spectrum β-lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. Antimicrob Agents Chemother 45:3355–3361. https://doi.org/10.1128/AAC.45.12.3355-3361.2001.
265. Dellus-Gur E, Toth-Petroczy A, Elias M, Tawfik DS. 2013. What makes a protein fold amenable to functional innovation? Fold polarity and stability trade-offs. J Mol Biol 425:2609–2621. https://doi.org/10.1016/j.jmb.2013.03.033.
266. Novais Â, Cantón R, Coque TM, Moya A, Baquero F, Galán JC. 2008. Mutational events in cefotaximase extended-spectrum β-lactamases of the CTX-M-1 cluster involved in ceftazidime resistance. Antimicrob Agents Chemother 52:2377–2382. https://doi.org/10.1128/AAC.01658-07.
267. Galán JC, González-Candelas F, Rolain JM, Cantón R. 2013. Antibiotics as selectors and accelerators of diversity in the mechanisms of resistance: from the resistome to genetic plasticity in the β-lactamases world. Front Microbiol 4:9. https://doi.org/10.3389/fmicb.2013.00009.
268. Traulsen A, Iwasa Y, Nowak MA. 2007. The fastest evolutionary trajectory. J Theor Biol 249:617–623. https://doi.org/10.1016/j.jtbi.2007.08.012.
269. Bloom JD, Lu Z, Chen D, Raval A, Venturelli OS, Arnold FH. 2007. Evolution favors protein mutational robustness in sufficiently large populations. BMC Biol 5:29. https://doi.org/10.1186/1741-7007-5-29.
270. Codoñer FM, Daròs JA, Solé RV, Elena SF. 2006. The fittest versus the flattest: experimental confirmation of the quasispecies effect with subviral pathogens. PLoS Pathog 2:e136. https://doi.org/10.1371/journal.ppat.0020136.
271. Wagner A. 2012. The role of robustness in phenotypic adaptation and innovation. Proc Biol Sci 279:1249–1258. https://doi.org/10.1098/rspb.2011.2293.
272. Kirby R. 1990. Evolutionary origin of aminoglycoside phosphotransferase resistance genes. J Mol Evol 30:489–492. https://doi.org/10.1007/BF02101103.
273. Nemec A, Dolzani L, Brisse S, Van Den Broek P, Dijkshoorn L. 2004. Diversity of aminoglycoside-resistance genes and their association with class 1 integrons among strains of pan-European *Acinetobacter baumannii* clones. J Med Microbiol 53:1233–1240. https://doi.org/10.1099/jmm.0.45716-0.
274. Allmansberger R, Bräu B, Piepersberg W. 1985. Genes for gentamicin-(3)-N-acetyl-transferases III and IV. Nucleotide sequences of three AAC(3)-III genes and evolutionary aspects. Mol Gen Genet 198:514–520. https://doi.org/10.1007/BF00332949.
275. García-León G, Salgado F, Oliveros JC, Sánchez MB, Martínez JL. 2014. Interplay between intrinsic and acquired resistance to quinolones in *Stenotrophomonas maltophilia*. Environ Microbiol 16:1282–1296. https://doi.org/10.1111/1462-2920.12408.
276. Riou M, Avrain L, Carbonnelle S, El Garch F, Pirnay JP, De Vos D, Plésiat P, Tulkens PM, Van Bambeke F. 2016. Increase of efflux-mediated resistance in *Pseudomonas aeruginosa* during antibiotic treatment in patients suffering from nosocomial pneumonia. Int J Antimicrob Agents 47:77–83. https://doi.org/10.1016/j.ijantimicag.2015.11.004.
277. Blair JMA, Bavro VN, Ricci V, Modi N, Cacciotto P, Kleinekathöfer U, Ruggerone P, Vargiu AV, Baylay AJ, Smith HE, Brandon Y, Galloway D, Piddock LJV. 2015. AcrB drug-binding pocket substitution confers clinically relevant resistance and altered substrate specificity. Proc Natl Acad Sci U S A 112:3511–3516. https://doi.org/10.1073/pnas.1419939112.
278. Blanco P, Corona F, Martínez JL. 2019. Involvement of the RND efflux pump transporter SmeH in the acquisition of resistance to ceftazidime in *Stenotrophomonas maltophilia*. Sci Rep 9:4917. https://doi.org/10.1038/s41598-019-41308-9.
279. de Lorenzo V. 2011. Beware of metaphors chasses and orthogonality in synthetic biology. Bioeng Bugs 2:3–7. https://doi.org/10.4161/bbug.2.1.13388.
280. Medrano-Soto A, Moreno-Hagelsieb G, Vinuesa P, Christen JA, Collado-Vides J. 2004. Successful lateral transfer requires codon usage compatibility between foreign genes and recipient genomes. Mol Biol Evol 21:1884–1894. https://doi.org/10.1093/molbev/msh202.
281. Amorós-Moya D, Bedhomme S, Hermann M, Bravo IG. 2010. Evolution in regulatory regions rapidly compensates the cost of nonoptimal codon usage. Mol Biol Evol 27:2141–2151. https://doi.org/10.1093/molbev/msq103.
282. Pelchovich G, Nadejda S, Dana A, Tuller T, Bravo IG, Gophna U. 2014. Ribosomal mutations affecting the translation of genes that use non-optimal codons. FEBS J 281:3701–3718. https://doi.org/10.1111/febs.12892.
283. Laarits T, Bordalo P, Lemos B. 2016. Genes under weaker stabilizing selection increase network evolvability and rapid regulatory adaptation to an environmental shift. J Evol Biol 29:1602–1616. https://doi.org/10.1111/jeb.12897.
284. Ramsay JP, Firth N. 2017. Diverse mobilization strategies facilitate transfer of non- conjugative mobile genetic elements. Curr Opin Microbiol 38:1–9. https://doi.org/10.1016/j.mib.2017.03.003.
285. Pang TY, Lercher MJ. 2017. Supra-operonic clusters of functionally related genes (SOCs) are a source of horizontal gene co-transfers. Sci Rep 7:40294. https://doi.org/10.1038/srep40294.
286. Harmer CJ, Pong CH, Hall RM. 2020. Structures bounded by directly-oriented members of the IS26 family are pseudo-compound transposons. Plasmid 111:102530. https://doi.org/10.1016/j.plasmid.2020.102530.
287. Siefert JL. 2009. Defining the mobilome. Methods Mol Biol 532:13–27. https://doi.org/10.1007/978-1-60327-853-9_2.
288. Razavi M, Kristiansson E, Flach CF, Larsson DGJ. 2020. The association between insertion sequences and antibiotic resistance genes. mSphere 5:e00418-20. https://doi.org/10.1128/mSphere.00418-20.
289. Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31:e00088-17. https://doi.org/10.1128/CMR.00088-17.
290. Escudero JA, Loot C, Nivina A, Mazel D. 2015. The integron: adaptation on demand, p 139–162. *In* Craig N, Chandler M, Gellert M, Lambowitz A, Rice P, Sandmeyer S (ed), Mobile DNA III. ASM Press, Washington, DC.
291. Lederberg J. 1952. Cell genetics and hereditary symbiosis. Physiol Rev 32:403–430. https://doi.org/10.1152/physrev.1952.32.4.403.
292. Watanabe T. 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol Rev 27:87–115. https://doi.org/10.1128/BR.27.1.87-115.1963.
293. Russo TA, Johnson JR. 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. J Infect Dis 181:1753–1754. https://doi.org/10.1086/315418.
294. Levy SB, Clowes R, Koenig E. 1981. Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Press, New York, NY.
295. Thomas CM. 2000. The horizontal gene pool. Harwood Academic Publishers, Amsterdam, Netherlands.

296. Couturier M, Bex F, Bergquist PL, Maas WK. 1988. Identification and classification of bacterial plasmids. Microbiol Rev 52:375–395. https://doi.org/10.1128/MR.52.3.375-395.1988.
297. Villa L, Carattoli A. 2020. Plasmid typing and classification. Methods Mol Biol 2075:309–321. https://doi.org/10.1007/978-1-4939-9877-7_22.
298. Lanza VF, Baquero F, Martínez JL, Ramos-Ruíz R, González-Zorn B, Andremont A, Sánchez-Valenzuela A, Ehrlich SD, Kennedy S, Ruppé E, van Schaik W, Willems RJ, de la Cruz F, Coque TM. 2018. In-depth resistome analysis by targeted metagenomics. Microbiome 6:11. https://doi.org/10.1186/s40168-017-0387-y.
299. Clewell DB, Weaver KE, Dunny GM, Coque TM, Francia MV, Hayes F. 2014. Extrachromosomal and mobile elements in enterococci: transmission, maintenance, and epidemiology, p 1–112. *In* Gilmore MS, Clewell DB, Ike Y, Shankar N (ed), Enterococci: from commensals to leading causes of drug resistant infection. Massachusetts Eye and Ear Infirmary, Boston, MA.
300. Garcillán-Barcia MP, Alvarado A, de la Cruz F. 2011. Identification of bacterial plasmids based on mobility and plasmid population biology. FEMS Microbiol Rev 35:936–956. https://doi.org/10.1111/j.1574-6976.2011.00291.x.
301. Francia MV, Varsaki A, Garcillán-Barcia MP, Latorre A, Drainas C, de la Cruz F. 2004. A classification scheme for mobilization regions of bacterial plasmids. FEMS Microbiol Rev 28:79–100. https://doi.org/10.1016/j.femsre.2003.09.001.
302. Redondo-Salvo S, Fernández-López R, Ruiz R, Vielva L, de Toro M, Rocha E, Garcillán-Barcia MP, de la Cruz F. 2020. Pathways for horizontal gene transfer in bacteria revealed by a global map of their plasmids. Nat Commun 11:3602. https://doi.org/10.1038/s41467-020-17278-2.
303. Souza V, Rocha M, Valera A, Eguiarte LE. 1999. Genetic structure of natural populations of *Escherichia coli* in wild hosts on different continents. Appl Environ Microbiol 65:3373–3385. https://doi.org/10.1128/AEM.65.8.3373-3385.1999.
304. León-Sampedro R, del Campo R, Rodriguez-Baños M, Lanza VF, Pozuelo MJ, Francés-Cuesta C, Tedim AP, Freitas AR, Novais C, Peixe L, Willems RJL, Corander J, González Candelas F, Baquero F, Coque TM. 2019. Phylogenomics of *Enterococcus faecalis* from wild birds: new insights into host-associated differences in core and accessory genomes of the species. Environ Microbiol 21:3046–3062. https://doi.org/10.1111/1462-2920.14702.
305. Johnson TJ, Wannemuehler Y, Johnson SJ, Stell AL, Doetkott C, Johnson JR, Kim KS, Spanjaard L, Nolan LK. 2008. Comparison of extraintestinal pathogenic *Escherichia coli* strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. Appl Environ Microbiol 74:7043–7050. https://doi.org/10.1128/AEM.01395-08.
306. De Gelder L, Ponciano JM, Joyce P, Top EM. 2007. Stability of a promiscuous plasmid in different hosts: no guarantee for a long-term relationship. Microbiology (Reading) 153:452–463. https://doi.org/10.1099/mic.0.2006/001784-0.
307. Bottery MJ, Wood AJ, Brockhurst MA. 2017. Adaptive modulation of antibiotic resistance through intragenomic coevolution. Nat Ecol Evol 1:1364–1369. https://doi.org/10.1038/s41559-017-0242-3.
308. San Millan A, MacLean RC. 2017. Fitness costs of plasmids: a limit to plasmid transmission, p 65–79. In Baquero F, Bouza E, Gutiérrez-Fuentes JA, Coque TM (ed), Microbial transmission. ASM Press, Washington, DC.
309. Harrison E, Guymer D, Spiers AJ, Paterson S, Brockhurst MA. 2015. Parallel compensatory evolution stabilizes plasmids across the parasitism-mutualism continuum. Curr Biol 25:2034–2039. https://doi.org/10.1016/j.cub.2015.06.024.
310. Sengupta M, Austin S. 2011. Prevalence and significance of plasmid maintenance functions in the virulence plasmids of pathogenic bacteria. Infect Immun 79:2502–2509. https://doi.org/10.1128/IAI.00127-11.
311. San Millan A, Heilbron K, MacLean RC. 2014. Positive epistasis between co-infecting plasmids promotes plasmid survival in bacterial populations. ISME J 8:601–612. https://doi.org/10.1038/ismej.2013.182.
312. Hall JPJ, Wood AJ, Harrison E, Brockhurst MA. 2016. Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. Proc Natl Acad Sci U S A 113:8260–8265. https://doi.org/10.1073/pnas.1600974113.
313. Hall JPJ, Brockhurst MA, Dytham C, Harrison E. 2017. The evolution of plasmid stability: are infectious transmission and compensatory evolution competing evolutionary trajectories? Plasmid 91:90–95. https://doi.org/10.1016/j.plasmid.2017.04.003.
314. Rodríguez-Beltrán J, Sørum V, Toll-Riera M, de la Vega C, Peña-Miller R, Millán ÁS. 2020. Genetic dominance governs the evolution and spread of mobile genetic elements in bacteria. Proc Natl Acad Sci U S A 117:15755–15762. https://doi.org/10.1073/pnas.2001240117.
315. Paul D, Bhattacharjee A, Bhattacharjee D, Dhar D, Maurya AP, Chakravarty A. 2017. Transcriptional analysis of bla NDM-1 and copy number alteration under carbapenem stress. Antimicrob Resist Infect Control 6:26. https://doi.org/10.1186/s13756-017-0183-2.
316. Blackwell GA, Doughty EL, Moran RA. 2021. Evolution and dissemination of L and M plasmid lineages carrying antibiotic resistance genes in diverse Gram-negative bacteria. Plasmid 113:102528. https://doi.org/10.1016/j.plasmid.2020.102528.
317. Popowska M, Krawczyk-Balska A. 2013. Broad-host-range IncP-1 plasmids and their resistance potential. Front Microbiol 4:44. https://doi.org/10.3389/fmicb.2013.00044.
318. Harmer CJ, Hall RM. 2015. The A to Z of A/C plasmids. Plasmid 80:63–82. https://doi.org/10.1016/j.plasmid.2015.04.003.
319. Fernández-López R, Garcillán-Barcia MP, Revilla C, Lázaro M, Vielva L, De La Cruz F. 2006. Dynamics of the IncW genetic backbone imply general trends in conjugative plasmid evolution. FEMS Microbiol Rev 30:942–966. https://doi.org/10.1111/j.1574-6976.2006.00042.x.
320. Johnson TJ, Lang KS. 2012. IncA/C plasmids: an emerging threat to human and animal health? Mob Genet Elements 2:55–58. https://doi.org/10.4161/mge.19626.
321. Villa L, García-Fernández A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother 65:2518–2529. https://doi.org/10.1093/jac/dkq347.
322. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A. 2012. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. Plasmid 68:43–50. https://doi.org/10.1016/j.plasmid.2012.03.001.
323. Fernandez-Lopez R, de Toro M, Moncalian G, Garcillan-Barcia MP, de la Cruz F. 2016. Comparative genomics of the conjugation region of F-like plasmids: five shades of F. Front Mol Biosci 3:71. https://doi.org/10.3389/fmolb.2016.00071.
324. Partridge SR. 2011. Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol Rev 35:820–855. https://doi.org/10.1111/j.1574-6976.2011.00277.x.
325. Douard G, Praud K, Cloeckaert A, Doublet B. 2010. The *Salmonella* genomic island 1 is specifically mobilized in trans by the IncA/C multidrug resistance plasmid family. PLoS One 5:e15302. https://doi.org/10.1371/journal.pone.0015302.
326. Carraro N, Rivard N, Ceccarelli D, Colwell RR, Burrus V. 2016. IncA/C conjugative plasmids mobilize a new family of multidrug resistance islands in clinical Vibrio cholerae non-O1/non-O139 isolates from Haiti. mBio 7:e00509-16. https://doi.org/10.1128/mBio.00509-16.
327. Schultz E, Barraud O, Madec J-Y, Haenni M, Cloeckaert A, Ploy M-C, Doublet B. 2017. Multidrug resistance *Salmonella* genomic island 1 in a *Morganella morganii* subsp. *morganii* human clinical isolate from France. mSphere 2:e00118-17. https://doi.org/10.1128/mSphere.00118-17.
328. Schubert S, Darlu P, Clermont O, Wieser A, Magistro G, Hoffmann C, Weinert K, Tenaillon O, Matic I, Denamur E. 2009. Role of intraspecies recombination in the spread of pathogenicity islands within the Escherichia coli species. PLoS Pathog 5:e1000257. https://doi.org/10.1371/journal.ppat.1000257.
329. Manson JM, Hancock LE, Gilmore MS. 2010. Mechanism of chromosomal transfer of *Enterococcus faecalis* pathogenicity island, capsule, antimicrobial resistance, and other traits. Proc Natl Acad Sci U S A 107:12269–-12274. https://doi.org/10.1073/pnas.1000139107.
330. Aziz RK, Breitbart M, Edwards RA. 2010. Transposases are the most abundant, most ubiquitous genes in nature. Nucleic Acids Res 38:4207–4217. https://doi.org/10.1093/nar/gkq140.
331. Roberts AP, Chandler M, Courvalin P, Guédon G, Mullany P, Pembroke T, Rood JI, Jeffery Smith C, Summers AO, Tsuda M, Berg DE. 2008. Revised nomenclature for transposable genetic elements. Plasmid 60:167–173. https://doi.org/10.1016/j.plasmid.2008.08.001.
332. Siguier P, Filée J, Chandler M. 2006. Insertion sequences in prokaryotic genomes. Curr Opin Microbiol 9:526–531. https://doi.org/10.1016/j.mib.2006.08.005.
333. Touchon M, Rocha EPC. 2008. From GC skews to wavelets: a gentle guide to the analysis of compositional asymmetries in genomic data. Biochimie 90:648–659. https://doi.org/10.1016/j.biochi.2007.09.015.
334. Yassine H, Bientz L, Cros J, Goret J, Bébéar C, Quentin C, Arpin C. 2015. Experimental evidence for IS1294b-mediated transposition of the

blaCMY-2 cephalosporinase gene in Enterobacteriaceae. J Antimicrob Chemother 70:697–700. https://doi.org/10.1093/jac/dku472.
335. Harmer CJ, Moran RA, Hall RM. 2014. Movement of IS26-associated antibiotic resistance genes occurs via a translocatable unit that includes a single IS26 and preferentially inserts adjacent to another IS26. mBio 5: e01801-14. https://doi.org/10.1128/mBio.01801-14.
336. Harmer CJ, Hall RM. 2016. IS26-mediated formation of transposons carrying antibiotic resistance genes. mSphere 1:e00038-16. https://doi.org/10.1128/mSphere.00038-16.
337. Blackwell GA, Nigro SJ, Hall RM. 2015. Evolution of AbGRI2-0, the progenitor of the AbGRI2 resistance island in global clone 2 of *Acinetobacter baumannii*. Antimicrob Agents Chemother 60:1421–1429. https://doi.org/10.1128/AAC.02662-15.
338. Reid CJ, Chowdhury PR, Djordjevic SP. 2015. Tn6026 and Tn6029 are found in complex resistance regions mobilised by diverse plasmids and chromosomal islands in multiple antibiotic resistant Enterobacteriaceae. Plasmid 80:127–137. https://doi.org/10.1016/j.plasmid.2015.04.005.
339. Beyrouthy R, Robin F, Delmas J, Gibold L, Dalmasso G, Dabboussi F, Hamzé M, Bonnet R. 2014. IS1R-mediated plasticity of IncL/M plasmids leads to the insertion of blaOXA-48 into the *Escherichia coli* chromosome. Antimicrob Agents Chemother 58:3785–3790. https://doi.org/10.1128/AAC.02669-14.
340. Ambrose SJ, Harmer CJ, Hall RM. 2018. Evolution and typing of IncC plasmids contributing to antibiotic resistance in Gram-negative bacteria. Plasmid 99:40–55. https://doi.org/10.1016/j.plasmid.2018.08.001.
341. Lee H, Doak TG, Popodi E, Foster PL, Tang H. 2016. Insertion sequence-caused large-scale rearrangements in the genome of *Escherichia coli*. Nucleic Acids Res 44:7109–7119. https://doi.org/10.1093/nar/gkw647.
342. Touchon M, Rocha EPC. 2007. Causes of insertion sequences abundance in prokaryotic genomes. Mol Biol Evol 24:969–981. https://doi.org/10.1093/molbev/msm014.
343. Bichsel M, Barbour AD, Wagner A. 2010. The early phase of a bacterial insertion sequence infection. Theor Popul Biol 78:278–288. https://doi.org/10.1016/j.tpb.2010.08.003.
344. Bichsel M, Barbour AD, Wagner A. 2013. Estimating the fitness effect of an insertion sequence. J Math Biol 66:95–114. https://doi.org/10.1007/s00285-012-0504-2.
345. Hartl DL, Sawyer SA. 1988. Why do unrelated insertion sequences occur together in the genome of *Escherichia coli*? Genetics 118:537–541. https://doi.org/10.1093/genetics/118.3.537.
346. Ooka T, Ogura Y, Asadulghani M, Ohnishi M, Nakayama K, Terajima J, Watanabe H, Hayashi T. 2009. Inference of the impact of insertion sequence (IS) elements on bacterial genome diversification through analysis of small-size structural polymorphisms in *Escherichia coli* O157 genomes. Genome Res 19:1809–1816. https://doi.org/10.1101/gr.089615.108.
347. Di Gregorio S, Fernandez S, Perazzi B, Bello N, Famiglietti A, Mollerach M. 2016. Increase in IS256 transposition in invasive vancomycin heteroresistant *Staphylococcus aureus* isolate belonging to ST100 and its derived VISA mutants. Infect Genet Evol 43:197–202. https://doi.org/10.1016/j.meegid.2016.05.001.
348. Rankin DJ, Bichsel M, Wagner A. 2010. Mobile DNA can drive lineage extinction in prokaryotic populations. J Evol Biol 23:2422–2431. https://doi.org/10.1111/j.1420-9101.2010.02106.x.
349. Stoebel DM, Dorman CJ. 2010. The effect of mobile element IS10 on experimental regulatory evolution in *Escherichia coli*. Mol Biol Evol 27:2105–2112. https://doi.org/10.1093/molbev/msq101.
350. Dolgin ES, Charlesworth B. 2006. The fate of transposable elements in asexual populations. Genetics 174:817–827. https://doi.org/10.1534/genetics.106.060434.
351. Wu Y, Aandahl RZ, Tanaka MM. 2015. Dynamics of bacterial insertion sequences: can transposition bursts help the elements persist? Theories and models. BMC Evol Biol 15:288. https://doi.org/10.1186/s12862-015-0560-5.
352. Nicolas E, Lambin M, Dandoy D, Galloy C, Nguyen N, Oger CA, Hallet B. 2015. The Tn3-family of replicative transposons. Microbiol Spectr 3:MDNA3-0060-2014. https://doi.org/10.1128/microbiolspec.MDNA3-0060-2014.
353. Cohen SN. 1976. Transposable genetic elements and plasmid evolution. Nature 263:731–733. https://doi.org/10.1038/263731a0.
354. Hedges RW, Jacob AE. 1974. Transposition of ampicillin resistance from RP4 to other replicons. Mol Gen Genet 132:31–40. https://doi.org/10.1007/BF00268228.
355. Heffron F, Sublett R, Hedges RW, Jacob A, Falkow S. 1975. Origin of the TEM beta lactamase gene found on plasmids. J Bacteriol 122:250–256. https://doi.org/10.1128/JB.122.1.250-256.1975.
356. Liebert CA, Hall RM, Summers AO. 1999. Transposon Tn21, flagship of the floating genome. Microbiol Mol Biol Rev 63:507–522. https://doi.org/10.1128/MMBR.63.3.507-522.1999.
357. Mindlin SZ, Petrova MA. 2017. On the origin and distribution of antibiotic resistance: permafrost bacteria studies. Mol Genet Microbiol Virol 32:169–179. https://doi.org/10.3103/S0891416817040048.
358. Partridge SR, Brown HJ, Stokes HW, Hall RM. 2001. Transposons Tn1696 and Tn21 and their integrons In4 and In2 have independent origins. Antimicrob Agents Chemother 45:1263–1270. https://doi.org/10.1128/AAC.45.4.1263-1270.2001.
359. Harmer CJ, Hamidian M, Hall RM. 2017. pIP40a, a type 1 IncC plasmid from 1969 carries the integrative element GIsul2 and a novel class II mercury resistance transposon. Plasmid 92:17–25. https://doi.org/10.1016/j.plasmid.2017.05.004.
360. Novais A, Cantón R, Valverde A, Machado E, Galán J-C, Peixe L, Carattoli A, Baquero F, Coque TM. 2006. Dissemination and persistence of blaCTX-M-9 are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn402 located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. Antimicrob Agents Chemother 50:2741–2750. https://doi.org/10.1128/AAC.00274-06.
361. Rinkel M, Hubert JC, Roux B, Lett MC. 1994. Identification of a new transposon Tn 5403 in a *Klebsiella pneumoniae* strain isolated from a polluted aquatic environment. Curr Microbiol 29:249–254. https://doi.org/10.1007/BF01577436.
362. Dziewit L, Baj J, Szuplewska M, Maj A, Tabin M, Czyzkowska A, Skrzypczyk G, Adamczuk M, Sitarek T, Stawinski P, Tudek A, Wanasz K, Wardal E, Piechucka E, Bartosik D. 2012. Insights into the transposable mobilome of *Paracoccus* spp. (Alphaproteobacteria). PLoS One 7: e32277. https://doi.org/10.1371/journal.pone.0032277.
363. Li Z, Craig NL, Peters JE. 2013. Transposon Tn7, p 1–32. *In* Roberts AP, Mullany P (ed), Bacterial integrative mobile genetic elements. CRC Press, Boca Raton, FL.
364. Parks AR, Peters JE. 2009. Tn*7* elements: engendering diversity from chromosomes to episomes. Plasmid 61:1–14. https://doi.org/10.1016/j.plasmid.2008.09.008.
365. Ghaly TM, Chow L, Asher AJ, Waldron LS, Gillings MR. 2017. Evolution of class 1 integrons: mobilization and dispersal via food-borne bacteria. PLoS One 12:e0179169. https://doi.org/10.1371/journal.pone.0179169.
366. Mazel D. 2006. Integrons: agents of bacterial evolution. Nat Rev Microbiol 4:608–620. https://doi.org/10.1038/nrmicro1462.
367. Peters JE. 2019. Targeted transposition with Tn7 elements: safe sites, mobile plasmids, CRISPR/Cas and beyond. Mol Microbiol 112:1635–1644. https://doi.org/10.1111/mmi.14383.
368. Petrovski S, Blackmore DW, Jackson KL, Stanisich VA. 2011. Mercury(II)-resistance transposons Tn*502* and Tn*512*, from *Pseudomonas* clinical strains, are structurally different members of the Tn*5053* family. Plasmid 65:58–64. https://doi.org/10.1016/j.plasmid.2010.08.003.
369. Delihas N. 2011. Impact of small repeat sequences on bacterial genome evolution. Genome Biol Evol 3:959–973. https://doi.org/10.1093/gbe/evr077.
370. Gillings MR, Labbate M, Sajjad A, Giguère NJ, Holley MP, Stokes HW. 2009. Mobilization of a Tn402-like class 1 integron with a novel cassette array via flanking miniature inverted-repeat transposable element-like structures. Appl Environ Microbiol 75:6002–6004. https://doi.org/10.1128/AEM.01033-09.
371. Poirel L, Carrër A, Pitout JD, Nordmann P. 2009. Integron mobilization unit as a source of mobility of antibiotic resistance genes. Antimicrob Agents Chemother 53:2492–2498. https://doi.org/10.1128/AAC.00033-09.
372. Szuplewska M, Ludwiczak M, Lyzwa K, Czarnecki J, Bartosik D. 2014. Mobility and generation of mosaic non-autonomous transposons by Tn3-Derived Inverted-repeat Miniature Elements (TIMEs). PLoS One 9: e105010. https://doi.org/10.1371/journal.pone.0105010.
373. Wailan AM, Sidjabat HE, Yam WK, Alikhan NF, Petty NK, Sartor AL, Williamson DA, Forde BM, Schembri MA, Beatson SA, Paterson DL, Walsh TR, Partridge SR. 2016. Mechanisms involved in acquisition of *bla*NDM genes by IncA/C2 and IncFIIY plasmids. Antimicrob Agents Chemother 60:4082–4088. https://doi.org/10.1128/AAC.00368-16.
374. Rivard N, Colwell RR, Burrus V. 2020. Antibiotic resistance in *Vibrio cholerae*: mechanistic insights from IncC plasmid-mediated dissemination of a novel family of genomic islands inserted at *trmE*. mSphere 5:e00748-20. https://doi.org/10.1128/mSphere.00748-20.

375. Guglielmini J, Quintais L, Garcillán-Barcia MP, de la Cruz F, Rocha EPC. 2011. The repertoire of ICE in prokaryotes underscores the unity, diversity, and ubiquity of conjugation. PLoS Genet 7:e1002222. https://doi.org/10.1371/journal.pgen.1002222.
376. Spagnoletti M, Ceccarelli D, Rieux A, Fondi M, Taviani E, Fani R, Colombo MM, Colwell RR, Balloux F. 2014. Acquisition and evolution of SXT-R391 integrative conjugative elements in the seventh-pandemic Vibrio cholerae lineage. mBio 5:e01356-14. https://doi.org/10.1128/mBio.01356-14.
377. Carraro N, Burrus V. 2015. The dualistic nature of integrative and conjugative elements. Mob Genet Elements 5:98–102. https://doi.org/10.1080/2159256X.2015.1102796.
378. Delavat F, Miyazaki R, Carraro N, Pradervand N, van der Meer JR. 2017. The hidden life of integrative and conjugative elements. FEMS Microbiol Rev 41:512–537. https://doi.org/10.1093/femsre/fux008.
379. Keen EC. 2015. A century of phage research: bacteriophages and the shaping of modern biology. Bioessays 37:6–9. https://doi.org/10.1002/bies.201400152.
380. Torres-Barceló C. 2018. The disparate effects of bacteriophages on antibiotic-resistant bacteria. Emerg Microbes Infect 7:168. https://doi.org/10.1038/s41426-018-0169-z.
381. Modi SR, Lee HH, Spina CS, Collins JJ. 2013. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature 499:219–222. https://doi.org/10.1038/nature12212.
382. Ross J, Topp E. 2015. Abundance of antibiotic resistance genes in bacteriophage following soil fertilization with dairy manure or municipal biosolids, and evidence for potential transduction. Appl Environ Microbiol 81:7905–7913. https://doi.org/10.1128/AEM.02363-15.
383. Enault F, Briet A, Bouteille L, Roux S, Sullivan MB, Petit MA. 2017. Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. ISME J 11:237–247. https://doi.org/10.1038/ismej.2016.90.
384. Halary S, Leigh JW, Cheaib B, Lopez P, Bapteste E. 2010. Network analyses structure genetic diversity in independent genetic worlds. Proc Natl Acad Sci U S A 107:127–132. https://doi.org/10.1073/pnas.0908978107.
385. Jiang W, Maniv I, Arain F, Wang Y, Levin BR, Marraffini LA. 2013. Dealing with the evolutionary downside of CRISPR immunity: bacteria and beneficial plasmids. PLoS Genet 9:e1003844. https://doi.org/10.1371/journal.pgen.1003844.
386. Lang AS, Zhaxybayeva O, Beatty JT. 2012. Gene transfer agents: phage-like elements of genetic exchange. Nat Rev Microbiol 10:472–482. https://doi.org/10.1038/nrmicro2802.
387. Heuer H, Abdo Z, Smalla K. 2008. Patchy distribution of flexible genetic elements in bacterial populations mediates robustness to environmental uncertainty. FEMS Microbiol Ecol 65:361–371. https://doi.org/10.1111/j.1574-6941.2008.00539.x.
388. Lenski RE, Barrick JE, Ofria C. 2006. Balancing robustness and evolvability. PLoS Biol 4:e428. https://doi.org/10.1371/journal.pbio.0040428.
389. Hernando-Amado S, Coque TM, Baquero F, Martínez JL. 2020. Antibiotic resistance: moving from individual health norms to social norms in One Health and Global Health. Front Microbiol 11:1914. https://doi.org/10.3389/fmicb.2020.01914.
390. Hernando-Amado S, Coque TM, Baquero F, Martínez JL. 2019. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nat Microbiol 4:1432–1442. https://doi.org/10.1038/s41564-019-0503-9.
391. Woegerbauer M, Bellanger X, Merlin C. 2020. Cell-free DNA: an underestimated source of antibiotic resistance gene dissemination at the interface between human activities and downstream environments in the context of wastewater reuse. Front Microbiol 11:671. https://doi.org/10.3389/fmicb.2020.00671.
392. Thomas CM, Nielsen KM. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 3:711–721. https://doi.org/10.1038/nrmicro1234.
393. Schatz D, Vardi A. 2018. Extracellular vesicles—new players in cell–cell communication in aquatic environments. Curr Opin Microbiol 43:148–154. https://doi.org/10.1016/j.mib.2018.01.014.
394. Biller SJ, Schubotz F, Roggensack SE, Thompson AW, Summons RE, Chisholm SW. 2014. Bacterial vesicles in marine ecosystems. Science 343:183–186. https://doi.org/10.1126/science.1243457.
395. García-Aljaro C, Ballesté E, Muniesa M. 2017. Beyond the canonical strategies of horizontal gene transfer in prokaryotes. Curr Opin Microbiol 38:95–105. https://doi.org/10.1016/j.mib.2017.04.011.
396. Lu Y, Zeng J, Wang L, Lan K, Shunmei E, Wang L, Xiao Q, Luo Q, Huang X, Huang B, Chen C. 2017. Antibiotics promote *Escherichia coli-Pseudomonas aeruginosa* conjugation through inhibiting quorum sensing. Antimicrob Agents Chemother 61:e01284-17. https://doi.org/10.1128/AAC.01284-17.
397. Lu J, Wang Y, Li J, Mao L, Nguyen SH, Duarte T, Coin L, Bond P, Yuan Z, Guo J. 2018. Triclosan at environmentally relevant concentrations promotes horizontal transfer of multidrug resistance genes within and across bacterial genera. Environ Int 121:1217–1226. https://doi.org/10.1016/j.envint.2018.10.040.
398. Xie WY, Shen Q, Zhao FJ. 2018. Antibiotics and antibiotic resistance from animal manures to soil: a review. Eur J Soil Sci 69:181–195. https://doi.org/10.1111/ejss.12494.
399. Kotnik T, Weaver JC. 2016. Abiotic gene transfer: rare or rampant? J Membr Biol 249:623–631. https://doi.org/10.1007/s00232-016-9897-y.
400. Rodríguez-Beltrán J, Rodríguez-Rojas A, Yubero E, Blázquez J. 2013. The animal food supplement sepiolite promotes a direct horizontal transfer of antibiotic resistance plasmids between bacterial species. Antimicrob Agents Chemother 57:2651–2653. https://doi.org/10.1128/AAC.02363-12.
401. Shaw LP, Wang AD, Dylus D, Meier M, Pogacnik G, Dessimoz C, Balloux F. 2020. The phylogenetic range of bacterial and viral pathogens of vertebrates. Mol Ecol 29:3361–3379. https://doi.org/10.1111/mec.15463.
402. Price LB, Hungate BA, Koch BJ, Davis GS, Liu CM. 2017. Colonizing opportunistic pathogens (COPs): the beasts in all of us. PLoS Pathog 13:e1006369. https://doi.org/10.1371/journal.ppat.1006369.
403. Matuszewska M, Murray GGR, Harrison EM, Holmes MA, Weinert LA. 2020. The evolutionary genomics of host specificity in Staphylococcus aureus. Trends Microbiol 28:465–477. https://doi.org/10.1016/j.tim.2019.12.007.
404. Torres C, Alonso CA, Ruiz-Ripa L, León-Sampedro R, del Campo R, Coque TM. 2018. Antimicrobial resistance in *Enterococcus* spp. of animal origin, p 185–227. *In* Schwartz S, Cavaco LM, Shen J, Aarestrup FM (ed), Antimicrobial resistance in bacteria from livestock and companion animals. ASM Press, Washington, DC.
405. Chopra I, O'Neill AJ, Miller K. 2003. The role of mutators in the emergence of antibiotic-resistant bacteria. Drug Resist Updat 6:137–145. https://doi.org/10.1016/S1368-7646(03)00041-4.
406. Minoia M, Gaillard M, Reinhard F, Stojanov M, Sentchilo V, Van Der Meer JR. 2008. Stochasticity and bistability in horizontal transfer control of a genomic island in *Pseudomonas*. Proc Natl Acad Sci U S A 105:20792–-20797. https://doi.org/10.1073/pnas.0806164106.
407. Domingues S, Harms K, Fricke WF, Johnsen PJ, da Silva GJ, Nielsen KM. 2012. Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species. PLoS Pathog 8:e1002837. https://doi.org/10.1371/journal.ppat.1002837.
408. Blokesch M. 2017. In and out—contribution of natural transformation to the shuffling of large genomic regions. Curr Opin Microbiol 38:22–29. https://doi.org/10.1016/j.mib.2017.04.001.
409. Veening JW, Blokesch M. 2017. Interbacterial predation as a strategy for DNA acquisition in naturally competent bacteria. Nat Rev Microbiol 15:629. https://doi.org/10.1038/nrmicro.2017.89.
410. Ringel PD, Hu D, Basler M. 2017. The role of type VI secretion system effectors in target cell lysis and subsequent horizontal gene transfer. Cell Rep 21:3927–3940. https://doi.org/10.1016/j.celrep.2017.12.020.
411. Croucher NJ, Mostowy R, Wymant C, Turner P, Bentley SD, Fraser C. 2016. Horizontal DNA transfer mechanisms of bacteria as weapons of intragenomic conflict. PLoS Biol 14:e1002394. https://doi.org/10.1371/journal.pbio.1002394.
412. Kohler V, Keller W, Grohmann E. 2019. Regulation of gram-positive conjugation. Front Microbiol 10:1134. https://doi.org/10.3389/fmicb.2019.01134.
413. Dalia AB, Seed KD, Calderwood SB, Camilli A. 2015. A globally distributed mobile genetic element inhibits natural transformation of *Vibrio cholerae*. Proc Natl Acad Sci U S A 112:10485–10490. https://doi.org/10.1073/pnas.1509097112.
414. León-Sampedro R, Fernández-De-Bobadilla MD, San Millán Á, Baquero F, Coque TM. 2019. Transfer dynamics of Tn*6648*, a composite integrative conjugative element generated by tandem accretion of Tn*5801* and Tn*6647* in *Enterococcus faecalis*. J Antimicrob Chemother 74:2517–2523. https://doi.org/10.1093/jac/dkz239.
415. Osborn AM, da Silva Tatley FM, Steyn LM, Pickup RW, Saunders JR. 2000. Mosaic plasmids and mosaic replicons: evolutionary lessons from the analysis of genetic diversity in IncFII-related replicons. Microbiology 146:2267–2275. https://doi.org/10.1099/00221287-146-9-2267.
416. Shintani M, Sanchez ZK, Kimbara K. 2015. Genomics of microbial plasmids: classification and identification based on replication and transfer

systems and host taxonomy. Front Microbiol 6:242. https://doi.org/10.3389/fmicb.2015.00242.
417. Huguet KT, Rivard N, Garneau D, Palanee J, Burrus V. 2020. Replication of the *Salmonella* genomic island 1 (SGI1) triggered by helper IncC conjugative plasmids promotes incompatibility and plasmid loss. PLoS Genet 16:e1008965. https://doi.org/10.1371/journal.pgen.1008965.
418. Garcillán-Barcia MP, de la Cruz F. 2008. Why is entry exclusion an essential feature of conjugative plasmids? Plasmid 60:1–18. https://doi.org/10.1016/j.plasmid.2008.03.002.
419. Koonin EV, Makarova KS, Wolf YI. 2017. Evolutionary genomics of defense systems in archaea and bacteria. Annu Rev Microbiol 71:233–261. https://doi.org/10.1146/annurev-micro-090816-093830.
420. Makarova KS, Wolf YI, Koonin EV. 2013. Comparative genomics of defense systems in archaea and bacteria. Nucleic Acids Res 41:4360–4377. https://doi.org/10.1093/nar/gkt157.
421. Makarova KS, Wolf YI, Snir S, Koonin EV. 2011. Defense islands in bacterial and archaeal genomes and prediction of novel defense systems. J Bacteriol 193:6039–6056. https://doi.org/10.1128/JB.05535-11.
422. Popa O, Dagan T. 2011. Trends and barriers to lateral gene transfer in prokaryotes. Curr Opin Microbiol 14:615–623. https://doi.org/10.1016/j.mib.2011.07.027.
423. Beiko RG, Harlow TJ, Ragan MA. 2005. Highways of gene sharing in prokaryotes. Proc Natl Acad Sci U S A 102:14332–14337. https://doi.org/10.1073/pnas.0504068102.
424. Alvarez-Ponce D, Lopez P, Bapteste E, Mcinerney JO. 2013. Gene similarity networks provide tools for understanding eukaryote origins and evolution. Proc Natl Acad Sci U S A 110:E1594–E1603. https://doi.org/10.1073/pnas.1211371110.
425. Davis JJ, Olsen GJ. 2011. Characterizing the native codon usages of a genome: an axis projection approach. Mol Biol Evol 28:211–221. https://doi.org/10.1093/molbev/msq185.
426. Oliveira PH, Touchon M, Rocha EPC. 2016. Regulation of genetic flux between bacteria by restriction-modification systems. Proc Natl Acad Sci U S A 113:5658–5663. https://doi.org/10.1073/pnas.1603257113.
427. Klümper U, Riber L, Dechesne A, Sannazzarro A, Hansen LH, Sørensen SJ, Smets BF. 2015. Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. ISME J 9:934–945. https://doi.org/10.1038/ismej.2014.191.
428. Norman A, Hansen LH, Sørensen SJ. 2009. Conjugative plasmids: vessels of the communal gene pool. Philos Trans R Soc Lond B Biol Sci 364:2275–2289. https://doi.org/10.1098/rstb.2009.0037.
429. Musovic S, Dechesne A, Sørensen J, Smets BF. 2010. Novel assay to assess permissiveness of a soil microbial community toward receipt of mobile genetic elements. Appl Environ Microbiol 76:4813–4818. https://doi.org/10.1128/AEM.02713-09.
430. Hall JPJ, Brockhurst MA, Harrison E, Sheffield S, Brockhurst MA. 2017. Sampling the mobile gene pool: innovation via horizontal gene transfer in bacteria. Philos Trans R Soc Lond B Biol Sci 372:20160424. https://doi.org/10.1098/rstb.2016.0424.
431. Baquero F, Lanza VF, Duval M, Coque TM. 2020. Ecogenetics of antibiotic resistance in *Listeria monocytogenes*. Mol Microbiol 113:570–579. https://doi.org/10.1111/mmi.14454.
432. Smillie C, Garcillán-Barcia MP, Francia MV, Rocha EPC, de la Cruz F. 2010. Mobility of plasmids. Microbiol Mol Biol Rev 74:434–452. https://doi.org/10.1128/MMBR.00020-10.
433. Dionisio F, Zilhão R, Gama JA. 2019. Interactions between plasmids and other mobile genetic elements affect their transmission and persistence. Plasmid 102:29–36. https://doi.org/10.1016/j.plasmid.2019.01.003.
434. Chavda KD, Chen L, Jacobs MR, Rojtman AD, Bonomo RA, Kreiswirth BN. 2015. Complete sequence of a *bla*KPC-harboring cointegrate plasmid isolated from *Escherichia coli*. Antimicrob Agents Chemother 59:2956–2959. https://doi.org/10.1128/AAC.00041-15.
435. Gama JA, Zilhão R, Dionisio F. 2018. Impact of plasmid interactions with the chromosome and other plasmids on the spread of antibiotic resistance. Plasmid 99:82–88. https://doi.org/10.1016/j.plasmid.2018.09.009.
436. Alonso-del Valle A, León-Sampedro R, Rodríguez-Beltrán J, Hernández-García M, Ruiz-Garbajosa P, Cantón R, San Millán Á, Spain M, Peña-Miller R. 2020. The distribution of plasmid fitness effects explains plasmid persistence in bacterial communities. bioRxiv 2020.08.01.230672.
437. Silva RF, Mendonça SCM, Carvalho LM, Reis AM, Gordo I, Trindade S, Dionisio F. 2011. Pervasive sign epistasis between conjugative plasmids and drug-resistance chromosomal mutations. PLoS Genet 7:e1002181. https://doi.org/10.1371/journal.pgen.1002181.
438. Gama JA, Zilhão R, Dionisio F. 2020. Plasmid interactions can improve plasmid persistence in bacterial populations. Front Microbiol 11:2033. https://doi.org/10.3389/fmicb.2020.02033.
439. Vogwill T, MacLean RC. 2015. The genetic basis of the fitness costs of antimicrobial resistance: a meta-analysis approach. Evol Appl 8:284–295. https://doi.org/10.1111/eva.12202.
440. Hülter NF, Wein T, Effe J, Garoña A, Dagan T. 2020. Intracellular competitions reveal determinants of plasmid evolutionary success. Front Microbiol 11:2062. https://doi.org/10.3389/fmicb.2020.02062.
441. Getino M, de la Cruz F. 2018. Natural and artificial strategies to control the conjugative transmission of plasmids, p 33–64. *In* Baquero F, Bouza E, Gutiérrez-Fuentes JA, Coque TM, (ed), Microbial transmission. ASM Press, Washington, DC.
442. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018.
443. Bouet JY, Funnell BE. 2019. Plasmid localization and partition in Enterobacteriaceae. EcoSal Plus 8:ESP-0003-2019. https://doi.org/10.1128/ecosalplus.ESP-0003-2019.
444. Cooper TF, Heinemann JA. 2005. Selection for plasmid post-segregational killing depends on multiple infection: evidence for the selection of more virulent parasites through parasite-level competition. Proc Biol Sci 272:403–410. https://doi.org/10.1098/rspb.2004.2921.
445. Penadés JR, Christie GE. 2015. The phage-inducible chromosomal islands: a family of highly evolved molecular parasites. Annu Rev Virol 2:181–201. https://doi.org/10.1146/annurev-virology-031413-085446.
446. Flament-Simon SC, de Toro M, Chuprikova L, Blanco M, Moreno-González J, Salas M, Blanco J, Redrejo-Rodríguez M. 2020. High diversity and variability of pipolins among a wide range of pathogenic Escherichia coli strains. Sci Rep 10:12452. https://doi.org/10.1038/s41598-020-69356-6.
447. Staley JT. 2006. The bacterial species dilemma and the genomic-phylogenetic species concept. Philos Trans R Soc Lond B Biol Sci 361:1899–1909. https://doi.org/10.1098/rstb.2006.1914.
448. Riley MA, Lizotte-Waniewski M. 2009. Population genomics and the bacterial species concept. Methods Mol Biol 532:367–377. https://doi.org/10.1007/978-1-60327-853-9_21.
449. Baquero F, Coque TM, Galán JC, Martinez JL. 2021. The origin of niches and species in the bacterial world. Front Microbiol 12:566. https://doi.org/10.3389/fmicb.2021.657986.
450. Levin BR. 1993. The accessory genetic elements of bacteria: existence conditions and (co)evolution. Curr Opin Genet Dev 3:849–854. https://doi.org/10.1016/0959-437X(93)90004-9.
451. Stalder T, Rogers LM, Renfrow C, Yano H, Smith Z, Top EM. 2017. Emerging patterns of plasmid-host coevolution that stabilize antibiotic resistance. Sci Rep 7:4853. https://doi.org/10.1038/s41598-017-04662-0.
452. Hughes JM, Lohman BK, Deckert GE, Nichols EP, Settles M, Abdo Z, Top EM. 2012. The role of clonal interference in the evolutionary dynamics of plasmid-host adaptation. mBio 3:e00077-12. https://doi.org/10.1128/mBio.00077-12.
453. Cooper TF, Paixão T, Heinemann JA. 2010. Within-host competition selects for plasmid encoded toxin-antitoxin systems. Proc Biol Sci 277:3149–3155. https://doi.org/10.1098/rspb.2010.0831.
454. Rankin DJ, Turner LA, Heinemann JA, Brown SP. 2012. The coevolution of toxin and antitoxin genes drives the dynamics of bacterial addiction complexes and intragenomic conflict. Proc Biol Sci 279:3706–3715. https://doi.org/10.1098/rspb.2012.0942.
455. Dimitriu T, Marchant L, Buckling A, Raymond B. 2019. Bacteria from natural populations transfer plasmids mostly towards their kin. Proc Biol Sci 286:20191110. https://doi.org/10.1098/rspb.2019.1110.
456. Nzabarushimana E, Tang H. 2018. Insertion sequence elements-mediated structural variations in bacterial genomes. Mob DNA 9:29. https://doi.org/10.1186/s13100-018-0134-3.
457. Gillings MR. 2014. Integrons: past, present, and future. Microbiol Mol Biol Rev 78:257–277. https://doi.org/10.1128/MMBR.00056-13.
458. Rowe-Magnus DA, Guerout AM, Mazel D. 2002. Bacterial resistance evolution by recruitment of super-integron gene cassettes. Mol Microbiol 43:1657–1669. https://doi.org/10.1046/j.1365-2958.2002.02861.x.
459. Podglajen I, Breuil J, Rohaut A, Monsempes C, Collatz E. 2001. Multiple mobile promoter regions for the rare carbapenem resistance gene of *Bacteroides fragilis*. J Bacteriol 183:3531–3535. https://doi.org/10.1128/JB.183.11.3531-3535.2001.
460. Hall RJ, Whelan FJ, McInerney JO, Ou Y, Domingo-Sananes MR. 2020. Horizontal gene transfer as a source of conflict and cooperation in

prokaryotes. Front Microbiol 11:1569. https://doi.org/10.3389/fmicb.2020.01569.
461. Bergstrom CT, Lipsitch M, Levin BR. 2000. Natural selection, infectious transfer and the existence conditions for bacterial plasmids. Genetics 155:1505–1519. https://doi.org/10.1093/genetics/155.4.1505.
462. Touchon M, Rocha EPC. 2016. Coevolution of the organization and structure of prokaryotic genomes. Cold Spring Harb Perspect Biol 8:a018168. https://doi.org/10.1101/cshperspect.a018168.
463. Osborn AM, Böltner D. 2002. When phage, plasmids, and transposons collide: genomic islands, and conjugative- and mobilizable-transposons as a mosaic continuum. Plasmid 48:202–212. https://doi.org/10.1016/s0147-619x(02)00117-8.
464. Pesesky MW, Tilley R, Beck DAC. 2019. Mosaic plasmids are abundant and unevenly distributed across prokaryotic taxa. Plasmid 102:10–18. https://doi.org/10.1016/j.plasmid.2019.02.003.
465. Rodríguez I, Novais Â, Lira F, Valverde A, Curião T, Martínez JL, Baquero F, Cantón R, Coque TM. 2015. Antibiotic-resistant *Klebsiella pneumoniae* and *Escherichia coli* high-risk clones and an IncFII(k) mosaic plasmid hosting Tn*1* (*bla*TEM-4) in isolates from 1990 to 2004. Antimicrob Agents Chemother 59:2904–2908. https://doi.org/10.1128/AAC.00296-15.
466. Lanza VF, Tedim AP, Martínez JL, Baquero F, Coque TM. 2015. The plasmidome of Firmicutes: impact on the emergence and the spread of resistance to antimicrobials. Microbiol Spectr 3:PLAS-0039-2014. https://doi.org/10.1128/microbiolspec.PLAS-0039-2014.
467. Croucher NJ, Coupland PG, Stevenson AE, Callendrello A, Bentley SD, Hanage WP. 2014. Diversification of bacterial genome content through distinct mechanisms over different timescales. Nat Commun 5:1–12. https://doi.org/10.1038/ncomms6471.
468. Cooper TF. 2007. Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. PLoS Biol 5:e225. https://doi.org/10.1371/journal.pbio.0050225.
469. Perron GG, Lee AEG, Wang Y, Huang WE, Barraclough TG. 2012. Bacterial recombination promotes the evolution of multi-drug-resistance in functionally diverse populations. Proc Biol Sci 279:1477–1484. https://doi.org/10.1098/rspb.2011.1933.
470. Shen P, Huang HV. 1986. Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. Genetics 112:441–457. https://doi.org/10.1093/genetics/112.3.441.
471. Kung SH, Retchless AC, Kwan JY, Almeida RPP. 2013. Effects of DNA size on transformation and recombination efficiencies in *Xylella fastidiosa*. Appl Environ Microbiol 79:1712–1717. https://doi.org/10.1128/AEM.03525-12.
472. Pál C, Papp B, Lercher MJ. 2005. Horizontal gene transfer depends on gene content of the host. Bioinformatics 21:ii222–ii223. https://doi.org/10.1093/bioinformatics/bti1136.
473. Pál C, Papp B, Lercher MJ. 2005. Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. Nat Genet 37:1372–1375. https://doi.org/10.1038/ng1686.
474. Popa O, Hazkani-Covo E, Landan G, Martin W, Dagan T. 2011. Directed networks reveal genomic barriers and DNA repair bypasses to lateral gene transfer among prokaryotes. Genome Res 21:599–609. https://doi.org/10.1101/gr.115592.110.
475. Tamminen M, Virta M, Fani R, Fondi M. 2012. Large-scale analysis of plasmid relationships through gene-sharing networks. Mol Biol Evol 29:1225–1240. https://doi.org/10.1093/molbev/msr292.
476. González-Torres P, Pryszcz LP, Santos F, Martínez-García M, Gabaldón T, Antón J. 2015. Interactions between closely related bacterial strains are revealed by deep transcriptome sequencing. Appl Environ Microbiol 81:8445–8456. https://doi.org/10.1128/AEM.02690-15.
477. Gardner A, West SA, Wild G. 2011. The genetical theory of kin selection. J Evol Biol 24:1020–1043. https://doi.org/10.1111/j.1420-9101.2011.02236.x.
478. Smith JM, Feil EJ, Smith NH. 2000. Population structure and evolutionary dynamics of pathogenic bacteria. Bioessays 22:1115–1122. https://doi.org/10.1002/1521-1878(200012)22:12<1115::AID-BIES9>3.0.CO;2-R.
479. Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG. 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 95:3140–3145. https://doi.org/10.1073/pnas.95.6.3140.
480. Corander J, Marttinen P, Sirén J, Tang J. 2008. Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics 9:539. https://doi.org/10.1186/1471-2105-9-539.
481. Gevers D, Cohan FM, Lawrence JG, Spratt BG, Coenye T, Feil EJ, Stackebrandt E, Van de Peer Y, Vandamme P, Thompson FL, Swings J. 2005. Re-evaluating prokaryotic species. Nat Rev Microbiol 3:733–739. https://doi.org/10.1038/nrmicro1236.
482. Woodford N, Turton JF, Livermore DM. 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 35:736–755. https://doi.org/10.1111/j.1574-6976.2011.00268.x.
483. Guzman Prieto AM, van Schaik W, Rogers MRC, Coque TM, Baquero F, Corander J, Willems RJL. 2016. Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? Front Microbiol 7:788. https://doi.org/10.3389/fmicb.2016.00788.
484. Van Valen L. 1977. The Red Queen. Am Nat 111:809–810. https://doi.org/10.1086/283213.
485. Stenseth NC, Smith JM. 1984. Coevolution in ecosystems: Red Queen evolution or stasis? Evolution 38:870–880. https://doi.org/10.2307/2408397.
486. Atwood KC, Schneider LK, Ryan FJ. 1951. Periodic selection in *Escherichia coli*. Proc Natl Acad Sci U S A 37:146–155. https://doi.org/10.1073/pnas.37.3.146.
487. Turrientes MC, Baquero F, Levin BR, Martínez JL, Ripoll A, González-Alba JM, Tobes R, Manrique M, Baquero MR, Rodríguez-Domínguez MJ, Cantón R, Galán JC. 2013. Normal mutation rate variants arise in a mutator (Mut S) *Escherichia coli* population. PLoS One 8:e72963. https://doi.org/10.1371/journal.pone.0072963.
488. Bonachela JA, Wortel MT, Stenseth NC. 2017. Eco-evolutionary Red Queen dynamics regulate biodiversity in a metabolite-driven microbial system. Sci Rep 7:17655. https://doi.org/10.1038/s41598-017-17774-4.
489. Brockhurst MA, Chapman T, King KC, Mank JE, Paterson S, Hurst GDD. 2014. Running with the Red Queen: the role of biotic conflicts in evolution. Proc R Soc B 281:20141382. https://doi.org/10.1098/rspb.2014.1382.
490. Elena SF, Cooper VS, Lenski RE. 1996. Punctuated evolution caused by selection of rare beneficial mutations. Science 272:1802–1804. https://doi.org/10.1126/science.272.5269.1802.
491. Baquero F, Lemonnier M. 2009. Generational coexistence and ancestor's inhibition in bacterial populations. FEMS Microbiol Rev 33:958–967. https://doi.org/10.1111/j.1574-6976.2009.00184.x.
492. Brisson D. 2018. Negative frequency-dependent selection is frequently confounding. Front Ecol Evol 6:10. https://doi.org/10.3389/fevo.2018.00010.
493. Dimitriu T, Medaney F, Amanatidou E, Forsyth J, Ellis RJ, Raymond B. 2019. Negative frequency dependent selection on plasmid carriage and low fitness costs maintains extended spectrum β-lactamases in *Escherichia coli*. Sci Rep 9:1–7. https://doi.org/10.1038/s41598-019-53575-7.
494. McNally A, Kallonen T, Connor C, Abudahab K, Aanensen DM, Horner C, Peacock SJ, Parkhill J, Croucher NJ, Corander J. 2019. Diversification of colonization factors in a multidrug-resistant *Escherichia coli* lineage evolving under negative frequency-dependent selection. mBio 10:e00644-19. https://doi.org/10.1128/mBio.00644-19.
495. Cohan FM. 2006. Towards a conceptual and operational union of bacterial systematics, ecology, and evolution. Philos Trans R Soc Lond B Biol Sci 361:1985–1996. https://doi.org/10.1098/rstb.2006.1918.
497. Carlson SM, Cunningham CJ, Westley PAH. 2014. Evolutionary rescue in a changing world. Trends Ecol Evol 29:521–530. https://doi.org/10.1016/j.tree.2014.06.005.
498. Bell G. 2017. Evolutionary rescue. Annu Rev Ecol Evol Syst 48:605–627. https://doi.org/10.1146/annurev-ecolsys-110316-023011.
499. Bell G, Gonzalez A. 2009. Evolutionary rescue can prevent extinction following environmental change. Ecol Lett 12:942–948. https://doi.org/10.1111/j.1461-0248.2009.01350.x.
500. Jalasvuori M, Penttinen R. 2017. What can evolutionary rescue tell us about the emergence of new resistant bacteria? Future Microbiol 12:731–733. https://doi.org/10.2217/fmb-2017-0079.
501. Uecker H, Hermisson J. 2016. The role of recombination in evolutionary rescue. Genetics 202:721–732. https://doi.org/10.1534/genetics.115.180299.
502. Barroso-Batista J, Sousa A, Lourenço M, Bergman M-L, Sobral D, Demengeot J, Xavier KB, Gordo I. 2014. The first steps of adaptation of Escherichia coli to the gut are dominated by soft sweeps. PLoS Genet 10:e1004182. https://doi.org/10.1371/journal.pgen.1004182.
503. Baquero F, Vicente MF, Pérez-Diaz JC. 1985. β-Lactam coselection of sensitive and TEM-1 β-lactamase-producing subpopulations in heterogeneous *Escherichia coli* colonies. J Antimicrob Chemother 15:151–157. https://doi.org/10.1093/jac/15.2.151.
504. Frost I, Smith WPJ, Mitri S, Millan AS, Davit Y, Osborne JM, Pitt-Francis JM, MacLean RC, Foster KR. 2018. Cooperation, competition and

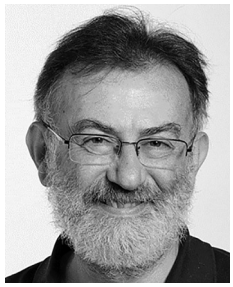
antibiotic resistance in bacterial colonies. ISME J 12:1582–1593. https://doi.org/10.1038/s41396-018-0090-4.
505. Conlin PL, Chandler JR, Kerr B. 2014. Games of life and death: antibiotic resistance and production through the lens of evolutionary game theory. Curr Opin Microbiol 21:35–44. https://doi.org/10.1016/j.mib.2014.09.004.
506. Reguera JA, Baquero F, Pérez-Díaz JC, Martínez JL. 1991. Factors determining resistance to β-lactam combined with β-lactamase inhibitors in *Escherichia coli*. J Antimicrob Chemother 27:569–575. https://doi.org/10.1093/jac/27.5.569.
507. Artemova T, Gerardin Y, Dudley C, Vega NM, Gore J. 2015. Isolated cell behavior drives the evolution of antibiotic resistance. Mol Syst Biol 11:822. https://doi.org/10.15252/msb.20145888.
508. Wright S. 1982. The shifting balance theory and macroevolution. Annu Rev Genet 16:1–19. https://doi.org/10.1146/annurev.ge.16.120182.000245.
509. Vos M, Didelot X. 2009. A comparison of homologous recombination rates in bacteria and archaea. ISME J 3:199–208. https://doi.org/10.1038/ismej.2008.93.
510. Baquero F, Lanza VF, Baquero MR, del Campo R, Bravo-Vázquez DA. 2019. Microcins in Enterobacteriaceae: peptide antimicrobials in the eco-active intestinal chemosphere. Front Microbiol 10:2261. https://doi.org/10.3389/fmicb.2019.02261.
511. Orskov F, Orskov I. 1992. *Escherichia coli* serotyping and disease in man and animals. Can J Microbiol 38:699–704. https://doi.org/10.1139/m92-115.
512. Gordon DM, O'Brien CL, Pavli P. 2015. Escherichia coli diversity in the lower intestinal tract of humans. Environ Microbiol Rep 7:642–648. https://doi.org/10.1111/1758-2229.12300.
513. Richter TKS, Hazen TH, Lam D, Coles CL, Seidman JC, You Y, Silbergeld EK, Fraser CM, Rasko DA. 2018. Temporal variability of *Escherichia coli* diversity in the gastrointestinal tracts of Tanzanian children with and without exposure to antibiotics. mSphere 3:e00558-18. https://doi.org/10.1128/mSphere.00558-18.
514. Rodríguez I, Figueiredo AS, Sousa M, Lanza V, Rodríguez C, Mingo P, Zamora J, Loza E, Brooks C, Cantón R, Baquero F, Coque T. 2021. A 21-year survey of *Escherichia coli* from bloodstream infections (BSIs) in a tertiary hospital reveals how community-hospital dynamics, influence local BSI rates, the trends of the B2 phylogroup and the STc131 pandemic clone. bioRxiv https://doi.org/10.1101/2020.04.10.034777.
496. Lefort A, Panhard X, Clermont O, Woerther P-L, Branger C, Mentré F, Fantin B, Wolff M, Denamur E, COLIBAFI Group. 2011. Host factors and portal of entry outweigh bacterial determinants to predict the severity of *Escherichia coli* bacteremia. J Clin Microbiol 49:777–783. https://doi.org/10.1128/JCM.01902-10.
515. Tedim AP, Ruiz-Garbajosa P, Corander J, Rodríguez CM, Cantón R, Willems RJ, Baquero F, Coque TM. 2015. Population biology of intestinal *Enterococcus* isolates from hospitalized and nonhospitalized individuals in different age groups. Appl Environ Microbiol 81:1820–1831. https://doi.org/10.1128/AEM.03661-14.
516. Weinberger DM, Malley R, Lipsitch M. 2011. Serotype replacement in disease after pneumococcal vaccination. Lancet 378:1962–1973. https://doi.org/10.1016/S0140-6736(10)62225-8.
517. Wyres KL, Lambertsen LM, Croucher NJ, McGee L, Von Gottberg A, Liñares J, Jacobs MR, Kristinsson KG, Beall BW, Klugman KP, Parkhill J, Hakenbeck R, Bentley SD, Brueggemann AB. 2013. Pneumococcal capsular switching: a historical perspective. J Infect Dis 207:439–449. https://doi.org/10.1093/infdis/jis703.
518. Erm P, Phillips BL. 2020. Evolution transforms pushed waves into pulled waves. Am Nat 195:E87–E99. https://doi.org/10.1086/707324.
519. Birzu G, Hallatschek O, Korolev KS. 2018. Fluctuations uncover a distinct class of traveling waves. Proc Natl Acad Sci U S A 115:E3645–E3654. https://doi.org/10.1073/pnas.1715737115.
520. Lundberg P, Ranta E, Ripa J, Kaitala V. 2000. Population variability in space and time. Trends Ecol Evol 15:460–464. https://doi.org/10.1016/s0169-5347(00)01981-9.
521. Ochocki BM, Miller TEX. 2017. Rapid evolution of dispersal ability makes biological invasions faster and more variable. Nat Commun 8:14315. https://doi.org/10.1038/ncomms14315.
522. Szücs M, Vahsen ML, Melbourne BA, Hoover C, Weiss-Lehman C, Hufbauer RA, Schoener TW. 2017. Rapid adaptive evolution in novel environments acts as an architect of population range expansion. Proc Natl Acad Sci U S A 114:13501–13506. https://doi.org/10.1073/pnas.1712934114.
523. Nowak MA, Tarnita CE, Antal T. 2010. Evolutionary dynamics in structured populations. Philos Trans R Soc Lond B Biol Sci 365:19–30. https://doi.org/10.1098/rstb.2009.0215.
524. Shine R, Brown GP, Phillips BL. 2011. An evolutionary process that assembles phenotypes through space rather than through time. Proc Natl Acad Sci U S A 108:5708–5711. https://doi.org/10.1073/pnas.1018989108.
525. Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x.
526. Baquero F. 2005. Evolution and the nature of time. Int Microbiol 8:81–91.
527. Tozzi A, Peters JF. 2018. What is it like to be "the same"? Prog Biophys Mol Biol 133:30–35. https://doi.org/10.1016/j.pbiomolbio.2017.10.005.
528. Dini-Andreote F, Van Elsas JD, Olff H, Salles JF. 2018. Dispersal-competition tradeoff in microbiomes in the quest for land colonization. Sci Rep 8:9451. https://doi.org/10.1038/s41598-018-27783-6.
529. Grilli J, Rogers T, Allesina S. 2016. Modularity and stability in ecological communities. Nat Commun 7:1–10. https://doi.org/10.1038/ncomms12031.
530. Mengistu H, Huizinga J, Mouret JB, Clune J. 2016. The evolutionary origins of hierarchy. PLoS Comput Biol 12:e1004829. https://doi.org/10.1371/journal.pcbi.1004829.
531. Haloin JR, Strauss SY. 2008. Interplay between ecological communities and evolution: review of feedbacks from microevolutionary to macroevolutionary scales. Ann N Y Acad Sci 1133:87–125. https://doi.org/10.1196/annals.1438.003.
532. Johnson MTJ, Stinchcombe JR. 2007. An emerging synthesis between community ecology and evolutionary biology. Trends Ecol Evol 22:250–257. https://doi.org/10.1016/j.tree.2007.01.014.
533. Moran NA, McLaughlin HJ, Sorek R. 2009. The dynamics and time scale of ongoing genomic erosion in symbiotic bacteria. Science 323:379–382. https://doi.org/10.1126/science.1167140.
534. Gasparrini AJ, Wang B, Sun X, Kennedy EA, Hernandez-Leyva A, Ndao IM, Tarr PI, Warner BB, Dantas G. 2019. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. Nat Microbiol 4:2285–2297. https://doi.org/10.1038/s41564-019-0550-2.
535. Baquero F, Nombela C. 2012. The microbiome as a human organ. Clin Microbiol Infect 18:2–4. https://doi.org/10.1111/j.1469-0691.2012.03916.x.
536. Plichta DR, Juncker AS, Bertalan M, Rettedal E, Gautier L, Varela E, Manichanh C, Fouqueray C, Levenez F, Nielsen T, Doré J, MacHado AMD, De Evgrafov MCR, Hansen T, Jørgensen T, Bork P, Guarner F, Pedersen O, Sommer MOA, Ehrlich SD, Sicheritz-Pontén T, Brunak S, Nielsen HB, Metagenomics of the Human Intestinal Tract (MetaHIT) Consortium. 2016. Transcriptional interactions suggest niche segregation among microorganisms in the human gut. Nat Microbiol 1:16152. https://doi.org/10.1038/nmicrobiol.2016.152.
537. Costea PI, Hildebrand F, Arumugam M, Bäckhed F, Blaser MJ, Bushman FD, de Vos WM, Ehrlich SD, Fraser CM, Hattori M, Huttenhower C, Jeffery IB, Knights D, Lewis JD, Ley RE, Ochman H, O'Toole PW, Quince C, Relman DA, Shanahan F, Sunagawa S, Wang J, Weinstock GM, Wu GD, Zeller G, Zhao L, Raes J, Knight R, Bork P. 2018. Enterotypes in the landscape of gut microbial community composition. Nat Microbiol 3:8–16. https://doi.org/10.1038/s41564-017-0072-8.
538. Raymond F, Ouameur AA, Déraspe M, Iqbal N, Gingras H, Dridi B, Leprohon P, Plante PL, Giroux R, Bérubé È, Frenette J, Boudreau DK, Simard JL, Chabot I, Domingo MC, Trottier S, Boissinot M, Huletsky A, Roy PH, Ouellette M, Bergeron MG, Corbeil J. 2016. The initial state of the human gut microbiome determines its reshaping by antibiotics. ISME J 10:707–720. https://doi.org/10.1038/ismej.2015.148.
539. Valverde A, Coque TM, García-San Miguel L, Baquero F, Cantón R. 2007. Complex molecular epidemiology of extended-spectrum β-lactamases in *Klebsiella pneumoniae*: a long-term perspective from a single institution in Madrid. J Antimicrob Chemother 61:64–72. https://doi.org/10.1093/jac/dkm403.
540. Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, Joakim Larsson DG, Johansson A. 2015. The human gut microbiome as a transporter of antibiotic resistance genes between continents. Antimicrob Agents Chemother 59:6551–6560. https://doi.org/10.1128/AAC.00933-15.
541. Rillig MC, Mansour I. 2017. Microbial ecology: community coalescence stirs things up. Curr Biol 27:R1280–R1282. https://doi.org/10.1016/j.cub.2017.10.027.
542. Pehrsson EC, Tsukayama P, Patel S, Mejía-Bautista M, Sosa-Soto G, Navarrete KM, Calderon M, Cabrera L, Hoyos-Arango W, Bertoli MT, Berg DE, Gilman RH, Dantas G. 2016. Interconnected microbiomes and

resistomes in low-income human habitats. Nature 533:212–216. https://doi.org/10.1038/nature17672.
543. Forslund K, Sunagawa S, Kultima JR, Mende DR, Arumugam M, Typas A, Bork P. 2013. Country-specific antibiotic use practices impact the human gut resistome. Genome Res 23:1163–1169. https://doi.org/10.1101/gr.155465.113.
544. Rodríguez-Verdugo A, Buckley J, Stapley J. 2017. The genomic basis of eco-evolutionary dynamics. Mol Ecol 26:1456–1464. https://doi.org/10.1111/mec.14045.
545. Martínez JL. 2018. Ecology and evolution of chromosomal gene transfer between environmental microorganisms and pathogens, p 141–160. *In* Baquero F, Bouza E, Gutiérrez-Fuentes JA, Coque TM (ed), Microbial transmission. ASM Press, Washington, DC.
546. Xiang Q, Chen QL, Zhu D, An XL, Yang XR, Su JQ, Qiao M, Zhu YG. 2018. Spatial and temporal distribution of antibiotic resistomes in a peri-urban area is associated significantly with anthropogenic activities. Environ Pollut 235:525–533. https://doi.org/10.1016/j.envpol.2017.12.119.
547. Szekeres E, Chiriac CM, Baricz A, Szőke-Nagy T, Lung I, Soran ML, Rudi K, Dragos N, Coman C. 2018. Investigating antibiotics, antibiotic resistance genes, and microbial contaminants in groundwater in relation to the proximity of urban areas. Environ Pollut 236:734–744. https://doi.org/10.1016/j.envpol.2018.01.107.
548. Slater FR, Bruce KD, Ellis RJ, Lilley AK, Turner SL. 2010. Determining the effects of a spatially heterogeneous selection pressure on bacterial population structure at the sub-millimetre scale. Microb Ecol 60:873–884. https://doi.org/10.1007/s00248-010-9687-5.
549. Baquero F. 2015. Causes and interventions: need of a multiparametric analysis of microbial ecobiology. Environ Microbiol Rep 7:13–14. https://doi.org/10.1111/1758-2229.12242.
550. Morrissey I, Oggioni MR, Knight D, Curiao T, Coque T, Kalkanci A, Martinez JL, Baldassarri L, Orefici G, Yetiş Ü, Rödger HJ, Visa P, Mora D, Leib S, Viti C, BIOHYPO Consortium. 2014. Evaluation of epidemiological cut-off values indicates that biocide resistant subpopulations are uncommon in natural isolates of clinically-relevant microorganisms. PLoS One 9:e86669. https://doi.org/10.1371/journal.pone.0086669.
551. Curiao T, Marchi E, Viti C, Oggioni MR, Baquero F, Martinez JL, Coque TM. 2015. Polymorphic variation in susceptibility and metabolism of triclosan-resistant mutants of *Escherichia coli* and *Klebsiella pneumoniae* clinical strains obtained after exposure to biocides and antibiotics. Antimicrob Agents Chemother 59:3413–3423. https://doi.org/10.1128/AAC.00187-15.
552. Curiao T, Marchi E, Grandgirard D, León-Sampedro R, Viti C, Leib SL, Baquero F, Oggioni MR, Martinez JL, Coque TM. 2016. Multiple adaptive routes of *Salmonella enterica* Typhimurium to biocide and antibiotic exposure. BMC Genomics 17:491. https://doi.org/10.1186/s12864-016-2778-z.
553. Booth S, Lewis RJ. 2019. Structural basis for the coordination of cell division with the synthesis of the bacterial cell envelope. Protein Sci 28:2042–2054. https://doi.org/10.1002/pro.3722.
554. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 447:345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032.
555. Bouki C, Venieri D, Diamadopoulos E. 2013. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review. Ecotoxicol Environ Saf 91:1–9. https://doi.org/10.1016/j.ecoenv.2013.01.016.
556. Yuan Q, Bin Guo MT, Wei WJ, Yang J. 2016. Reductions of bacterial antibiotic resistance through five biological treatment processes treated municipal wastewater. Environ Sci Pollut Res Int 23:19495–19503. https://doi.org/10.1007/s11356-016-7048-8.
557. Aydin S, Ince B, Ince O. 2015. Development of antibiotic resistance genes in microbial communities during long-term operation of anaerobic reactors in the treatment of pharmaceutical wastewater. Water Res 83:337–344. https://doi.org/10.1016/j.watres.2015.07.007.
558. Du B, Yang Q, Li X, Yuan W, Chen Y, Wang R. 2019. Impacts of long-term exposure to tetracycline and sulfamethoxazole on the sludge granules in an anoxic-aerobic wastewater treatment system. Sci Total Environ 684:67–77. https://doi.org/10.1016/j.scitotenv.2019.05.313.
559. Bengtsson-Palme J, Hammarén R, Pal C, Östman M, Björlenius B, Flach CF, Fick J, Kristiansson E, Tysklind M, Larsson DGJ. 2016. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Sci Total Environ 572:697–712. https://doi.org/10.1016/j.scitotenv.2016.06.228.
560. Goulas A, Belhadi D, Descamps A, Andremont A, Benoit P, Courtois S, Dagot C, Grall N, Makowski D, Nazaret S, Nélieu S, Patureau D, Petit F, Roose-Amsaleg C, Vittecoq M, Livoreil B, Laouénan C. 2020. How effective are strategies to control the dissemination of antibiotic resistance in the environment? A systematic review. Environ Evid 9:1–32.
561. Martínez JL, Baquero F. 2002. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev 15:647–679. https://doi.org/10.1128/cmr.15.4.647-679.2002.
562. Skwark MJ, Croucher NJ, Puranen S, Chewapreecha C, Pesonen M, Xu YY, Turner P, Harris SR, Beres SB, Musser JM, Parkhill J, Bentley SD, Aurell E, Corander J. 2017. Interacting networks of resistance, virulence and core machinery genes identified by genome-wide epistasis analysis. PLoS Genet 13:e1006508. https://doi.org/10.1371/journal.pgen.1006508.
563. Pérez-Gallego M, Torrens G, Castillo-Vera J, Moya B, Zamorano L, Cabot G, Hultenby K, Albertí S, Mellroth P, Henriques-Normark B, Normark S, Oliver A, Juan C. 2016. Impact of AmpC derepression on fitness and virulence: the mechanism or the pathway? mBio 7:e01783-16. https://doi.org/10.1128/mBio.01783-16.
564. Geisinger E, Isberg RR. 2017. Interplay between antibiotic resistance and virulence during disease promoted by multidrug-resistant bacteria. J Infect Dis 215:S9–S17. https://doi.org/10.1093/infdis/jiw402.
565. Sánchez P, Linares JF, Ruiz-Díez B, Campanario E, Navas A, Baquero F, Martínez JL. 2002. Fitness of in vitro selected *Pseudomonas aeruginosa nalB* and *nfxB* multidrug resistant mutants. J Antimicrob Chemother 50:657–664. https://doi.org/10.1093/jac/dkf185.
566. Datta S, Roy S, Chatterjee S, Saha A, Sen B, Pal T, Som T, Basu S. 2014. A five-year experience of carbapenem resistance in enterobacteriaceae causing neonatal septicaemia: predominance of NDM-1. PLoS One 9:e112101. https://doi.org/10.1371/journal.pone.0112101.
567. Johnson JR, Johnston B, Kuskowski MA, Colodner R, Raz R. 2005. Spontaneous conversion to quinolone and fluoroquinolone resistance among wild-type *Escherichia coli* isolates in relation to phylogenetic background and virulence genotype. Antimicrob Agents Chemother 49:4739–4744. https://doi.org/10.1128/AAC.49.11.4739-4744.2005.
568. Vila J, Simon K, Ruiz J, Horcajada JP, Velasco M, Barranco M, Moreno A, Mensa J. 2002. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? J Infect Dis 186:1039–1042. https://doi.org/10.1086/342955.
569. Hershow RC, Khayr WF, Smith NL. 1992. A comparison of clinical virulence of nosocomially acquired methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* infections in a university hospital. Infect Control Hosp Epidemiol 13:587–593. https://doi.org/10.1086/646433.
570. Yokoyama M, Stevens E, Laabei M. 2018. Epistasis analysis uncovers hidden antibiotic resistance-associated fitness costs hampering the evolution of MRSA. Genome Biol 19:1–12.
571. Morjaria S, Schluter J, Taylor BP, Littmann ER, Carter RA, Fontana E, Peled JU, van den Brink MRM, Xavier JB, Taur Y. 2019. Antibiotic-induced shifts in fecal microbiota density and composition during hematopoietic stem cell transplantation. Infect Immun 87:e00206-19. https://doi.org/10.1128/IAI.00206-19.
572. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, Van Den Brink MRM, Pamer EG. 2012. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 55:905–914. https://doi.org/10.1093/cid/cis580.
573. Lobkovsky AE, Wolf YI, Koonin EV. 2011. Predictability of evolutionary trajectories in fitness landscapes. PLoS Comput Biol 7:e1002302. https://doi.org/10.1371/journal.pcbi.1002302.
574. Furusawa C, Horinouchi T, Maeda T. 2018. Toward prediction and control of antibiotic-resistance evolution. Curr Opin Biotechnol 54:45–49. https://doi.org/10.1016/j.copbio.2018.01.026.
575. De Visser JAGM, Krug J. 2014. Empirical fitness landscapes and the predictability of evolution. Nat Rev Genet 15:480–490. https://doi.org/10.1038/nrg3744.
576. Lenski RE. 1991. Quantifying fitness and gene stability in microorganisms. Biotechnology 15:173–192. https://doi.org/10.1016/b978-0-409-90199-3.50015-2.
577. Travisano M, Vasi F, Lenski R. 1995. Long-term experimental evolution in *Escherichia coli*. III. variation among replicate populations in correlated responses to novel environments. Evolution 49:189–200. https://doi.org/10.1111/j.1558-5646.1995.tb05970.x.
578. Elena SF, Lenski RE. 2003. Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. Nat Rev Genet 4:457–469. https://doi.org/10.1038/nrg1088.

579. Fox JW, Lenski RE. 2015. From here to eternity—the theory and practice of a really long experiment. PLoS Biol 13:e1002185. https://doi.org/10.1371/journal.pbio.1002185.
580. Lenski RE, Wiser MJ, Ribeck N, Blount ZD, Nahum JR, Jeffrey Morris J, Zaman L, Turner CB, Wade BD, Maddamsetti R, Burmeister AR, Baird EJ, Bundy J, Grant NA, Card KJ, Rowles M, Weatherspoon K, Papoulis SE, Sullivan R, Clark C, Mulka JS, Hajela N. 2015. Sustained fitness gains and variability in fitness trajectories in the long-term evolution experiment with *Escherichia coli*. Proc Biol Sci 282:20152292. https://doi.org/10.1098/rspb.2015.2292.
581. Cooper VS, Schneider D, Blot M, Lenski RE. 2001. Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B. J Bacteriol 183:2834–2841. https://doi.org/10.1128/JB.183.9.2834-2841.2001.
582. Woods RJ, Barrick JE, Cooper TF, Shrestha U, Kauth MR, Lenski RE. 2011. Second-order selection for evolvability in a large *Escherichia coli* population. Science 331:1433–1436. https://doi.org/10.1126/science.1198914.
583. Blount ZD, Borland CZ, Lenski RE. 2008. Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. Proc Natl Acad Sci U S A 105:7899–7906. https://doi.org/10.1073/pnas.0803151105.
584. Blount ZD, Barrick JE, Davidson CJ, Lenski RE. 2012. Genomic analysis of a key innovation in an experimental *Escherichia coli* population. Nature 489:513–518. https://doi.org/10.1038/nature11514.
585. Blount ZD. 2016. A case study in evolutionary contingency. Stud Hist Philos Biol Biomed Sci 58:82–92. https://doi.org/10.1016/j.shpsc.2015.12.007.
586. Blount ZD, Lenski RE, Losos JB. 2018. Contingency and determinism in evolution: replaying life's tape. Science 362:eaam5979. https://doi.org/10.1126/science.aam5979.
587. Fumasoni M. 2020. Tell me where you've been and i'll tell you how you'll evolve. mBio 11:e02043-20. https://doi.org/10.1128/mBio.02043-20.
588. Baym M, Lieberman TD, Kelsic ED, Chait R, Gross R, Yelin I, Kishony R. 2016. Spatiotemporal microbial evolution on antibiotic landscapes. Science 353:1147–1151. https://doi.org/10.1126/science.aag0822.
589. Blanquart F, Bataillon T. 2016. Epistasis and the structure of fitness landscapes: are experimental fitness landscapes compatible with Fisher's geometric model? Genetics 203:847–862. https://doi.org/10.1534/genetics.115.182691.
590. Du Plessis L, Leventhal GE, Bonhoeffer S. 2016. How good are statistical models at approximating complex fitness landscapes? Mol Biol Evol 33:2454–2468. https://doi.org/10.1093/molbev/msw097.
591. Plucain J, Suau A, Cruveiller S, Médigue C, Schneider D, Le Gac M. 2016. Contrasting effects of historical contingency on phenotypic and genomic trajectories during a two-step evolution experiment with bacteria. BMC Evol Biol 16:86. https://doi.org/10.1186/s12862-016-0662-8.
592. Paegel BM. 2010. Microfluidic landscapes for evolution. Curr Opin Chem Biol 14:568–573. https://doi.org/10.1016/j.cbpa.2010.07.023.
593. Ripoll A, Baquero F, Novais Aõ, Rodríguez-Domínguez MJ, Turrientes MC, Cantón R, Galán JC. 2011. In vitro selection of variants resistant to β-lactams plus β-lactamase inhibitors in CTX-M β-lactamases: predicting the in vivo scenario? Antimicrob Agents Chemother 55:4530–4536. https://doi.org/10.1128/AAC.00178-11.
594. Shcherbakov D, Akbergenov R, Matt T, Sander P, Andersson DI, Böttger EC. 2010. Directed mutagenesis of *Mycobacterium smegmatis* 16S rRNA to reconstruct the in vivo evolution of aminoglycoside resistance in *Mycobacterium tuberculosis*. Mol Microbiol 77:830–840. https://doi.org/10.1111/j.1365-2958.2010.07218.x.
595. Gallet R, Cooper TF, Elena SF, Lenormand T. 2012. Measuring selection coefficients below 10^{-3}: method, questions, and prospects. Genetics 190:175–186. https://doi.org/10.1534/genetics.111.133454.
596. Wiser MJ, Lenski RE. 2015. A comparison of methods to measure fitness in *Escherichia coli*. PLoS One 10:e0126210. https://doi.org/10.1371/journal.pone.0126210.
597. Ram Y, Dellus-Gur E, Bibi M, Karkare K, Obolski U, Feldman MW, Cooper TF, Berman J, Hadany L. 2019. Predicting microbial growth in a mixed culture from growth curve data. Proc Natl Acad Sci U S A 116:14698–-14707. https://doi.org/10.1073/pnas.1902217116.
598. Buckling A, Craig MacLean R, Brockhurst MA, Colegrave N. 2009. The *Beagle* in a bottle. Nature 457:824–829. https://doi.org/10.1038/nature07892.
599. Toprak E, Veres A, Yildiz S, Pedraza JM, Chait R, Paulsson J, Kishony R. 2013. Building a morbidostat: an automated continuous-culture device for studying bacterial drug resistance under dynamically sustained drug inhibition. Nat Protoc 8:555–567. https://doi.org/10.1038/nprot.nprot.2013.021.
600. Sepkoski D. 2016. "Replaying Life's Tape": simulations, metaphors, and historicity in Stephen Jay Gould's view of life. Stud Hist Philos Biol Biomed Sci 58:73–81. https://doi.org/10.1016/j.shpsc.2015.12.009.
601. True JR, Haag ES. 2001. Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119. https://doi.org/10.1046/j.1525-142x.2001.003002109.x.
602. Powell R, Mariscal C. 2015. Convergent evolution as natural experiment: the tape of life reconsidered. Interface Focus 5:20150040. https://doi.org/10.1098/rsfs.2015.0040.
603. Lobkovsky AE, Koonin EV. 2012. Replaying the tape of life: quantification of the predictability of evolution. Front Genet 3:246. https://doi.org/10.3389/fgene.2012.00246.
604. Szendro IG, Franke J, De Visser JAGM, Krug J. 2013. Predictability of evolution depends nonmonotonically on population size. Proc Natl Acad Sci U S A 110:571–576. https://doi.org/10.1073/pnas.1213613110.
605. Achaz G, Rodriguez-Verdugo A, Gaut BS, Tenaillon O. 2014. The reproducibility of adaptation in the light of experimental evolution with whole genome sequencing. Adv Exp Med Biol 781:211–231. https://doi.org/10.1007/978-94-007-7347-9_11.
606. Boucher JI, Cote P, Flynn J, Jiang L, Laban A, Mishra P, Roscoe BP, Bolon DNA. 2014. Viewing protein fitness landscapes through a next-gen lens. Genetics 198:461–471. https://doi.org/10.1534/genetics.114.168351.
607. Anderson R, May R. 1991. Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford, United Kingdom.
608. Lipsitch M, Bergstrom CT, Levin BR. 2000. The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. Proc Natl Acad Sci U S A 97:1938–1943. https://doi.org/10.1073/pnas.97.4.1938.
609. Blanquart F. 2019. Evolutionary epidemiology models to predict the dynamics of antibiotic resistance. Evol Appl 12:365–383. https://doi.org/10.1111/eva.12753.
610. Leclerc QJ, Lindsay JA, Knight GM. 2019. Mathematical modelling to study the horizontal transfer of antimicrobial resistance genes in bacteria: current state of the field and recommendations. J R Soc Interface 16:20190260. https://doi.org/10.1098/rsif.2019.0260.
611. Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/BF01731581.
612. Schuster P. 2011. Mathematical modeling of evolution. Solved and open problems. Theory Biosci 130:71–89. https://doi.org/10.1007/s12064-010-0110-z.
613. Brenner K, You L, Arnold FH. 2008. Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol 26:483–489. https://doi.org/10.1016/j.tibtech.2008.05.004.
614. Song H, Ding MZ, Jia XQ, Ma Q, Yuan YJ. 2014. Synthetic microbial consortia: from systematic analysis to construction and applications. Chem Soc Rev 43:6954–6981. https://doi.org/10.1039/c4cs00114a.
615. Goldman RP, Brown SP. 2009. Making sense of microbial consortia using ecology and evolution. Trends Biotechnol 27:3–4. https://doi.org/10.1016/j.tibtech.2008.10.003.
616. Mee MT, Wang HH. 2012. Engineering ecosystems and synthetic ecologies. Mol Biosyst 8:2470–2483. https://doi.org/10.1039/c2mb25133g.
617. Tanouchi Y, Smith RP, You L. 2012. Engineering microbial systems to explore ecological and evolutionary dynamics. Curr Opin Biotechnol 23:791–797. https://doi.org/10.1016/j.copbio.2012.01.006.
618. Nielsen AAK, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, Ross D, Densmore D, Voigt CA. 2016. Genetic circuit design automation. Science 352:aac7341. https://doi.org/10.1126/science.aac7341.
619. Großkopf T, Consuegra J, Gaffé J, Willison JC, Lenski RE, Soyer OS, Schneider D. 2016. Metabolic modelling in a dynamic evolutionary framework predicts adaptive diversification of bacteria in a long-term evolution experiment. BMC Evol Biol 16:163. https://doi.org/10.1186/s12862-016-0733-x.
620. Jaffe AL, Corel E, Pathmanathan JS, Lopez P, Bapteste E. 2016. Bipartite graph analyses reveal interdomain LGT involving ultrasmall prokaryotes and their divergent, membrane-related proteins. Environ Microbiol 18:5072–5081. https://doi.org/10.1111/1462-2920.13477.
621. Kelly S, Maini PK. 2013. DendroBLAST: approximate phylogenetic trees in the absence of multiple sequence alignments. PLoS One 8:e58537. https://doi.org/10.1371/journal.pone.0058537.
622. Andrade RFS, Rocha-Neto IC, Santos LBL, de Santana CN, Diniz MVC, Lobão TP, Goés-Neto A, Pinho STR, El-Hani CN. 2011. Detecting network communities: an application to phylogenetic analysis. PLoS Comput Biol 7:e1001131. https://doi.org/10.1371/journal.pcbi.1001131.

623. Atkinson HJ, Morris JH, Ferrin TE, Babbitt PC. 2009. Using sequence similarity networks for visualization of relationships across diverse protein superfamilies. PLoS One 4:e4345. https://doi.org/10.1371/journal.pone.0004345.
624. Fischer EK, Ghalambor CK, Hoke KL. 2016. Can a network approach resolve how adaptive vs nonadaptive plasticity impacts evolutionary trajectories? Integr Comp Biol 56:877–888. https://doi.org/10.1093/icb/icw087.
625. Fischer EK, Ghalambor CK, Hoke KL. 2016. Plasticity and evolution in correlated suites of traits. J Evol Biol 29:991–1002. https://doi.org/10.1111/jeb.12839.
626. Rozenberg G, Salomaa A, Paun G. 2010. The Oxford handbook of membrane computing. Oxford University Press, Oxford, United Kingdom.
627. Campos M, Capilla R, Naya F, Futami R, Coque T, Moya A, Fernandez-Lanza V, Cantón R, Sempere JM, Llorens C, Baquero F. 2019. Simulating multilevel dynamics of antimicrobial resistance in a membrane computing model. mBio 10:e02460-18. https://doi.org/10.1128/mBio.02460-18.
628. Baquero F, Campos M, Llorens C, Sempere JM. 2018. A model of antibiotic resistance evolution dynamics through P systems with active membranes and communication rules, p 33–44. *In* Graciani C, Riscos-Núñez A, Päun G, Rozenberg G, Salomaa A (ed), Lecture notes in computer science. Springer, Cham, Switzerland.
629. Martínez JL, Baquero F. 2014. Emergence and spread of antibiotic resistance: setting a parameter space. Ups J Med Sci 119:68–77.
630. Darimont CT, Carlson SM, Kinnison MT, Paquet PC, Reimchen TE, Wilmers CC. 2009. Human predators outpace other agents of trait change in the wild. Proc Natl Acad Sci U S A 106:952–954. https://doi.org/10.1073/pnas.0809235106.
631. Braun M. 2003. On some properties of the multiple pendulum. Arch Appl Mech 72:899–910. https://doi.org/10.1007/s00419-002-0263-4.
632. Baquero F, Moya A. 2012. Intelligibility in microbial complex systems: Wittgenstein and the score of life. Front Cell Infect Microbiol 2:88. https://doi.org/10.3389/fcimb.2012.00088.
633. Feil EJ, Spratt BG. 2001. Recombination and the population structures of bacterial pathogens. Annu Rev Microbiol 55:561–590. https://doi.org/10.1146/annurev.micro.55.1.561.
634. Turing AM. 1952. The chemical basis of morphogenesis. *Philos Trans R Soc 237:37–72.*
635. Caron V, Ede FJ, Sunnucks P. 2014. Unravelling the paradox of loss of genetic variation during invasion: superclones may explain the success of a clonal invader. *PLoS One 9:e97744. https://doi.org/10.1371/journal.pone.0097744*.
636. Ogbunugafor CB, Eppstein MJ. 2017. Competition along trajectories governs adaptation rates towards antimicrobial resistance. Nat Ecol Evol 1:64. https://doi.org/10.1038/s41559-016-0064.
637. Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, Endtz HP, Cravioto A, Ali SI, Nakaya T, Horii T, Iida T, Alam M. 2011. Gut microbiota of healthy and malnourished children in Bangladesh. Front Microbiol 2:228.

F. Baquero, M.D., Ph.D., graduated in medicine at the Complutensis University in Madrid, specializing in clinical microbiology, and obtained his doctorate in 1973 at the Autonomous University in Madrid. He completed postdoctoral courses at the Pasteur Institute, Paris (1973–1974). From 1977 to 2008, he was Director of the Department of Microbiology at the Ramón y Cajal University Hospital in Madrid. He was subsequently Scientific Director of the Ramón y Cajal Health Research Institute (IRYCIS) (2008–2015). He was an associated scientist at the Astrobiology National Center (INTA-NASA). He has been a Research Professor in Microbial Evolution since 2008 and was the Director of the Division of Microbial Biology and Evolution of Microorganisms at IRYCIS. He worked on evolution of antibiotic resistance for 40 years, from 1995 in close interaction with Emory University. He has more than 500 publications in PubMed-referenced journals and published several books (e.g., *Evolutionary Biology of Bacterial Pathogens*, ASM Press). He obtained the ICAAC-ASM Award (2000) and is a member of the European and American Academy of Microbiology.



J. L. Martinez is a chemist by formation and microbiologist by career. He was Research Fellow at the Department of Microbiology at the Ramón y Cajal Hospital, Madrid, and the Imperial Cancer Research Foundation (UK). He is currently Full Research Professor at the National Biotechnology Center of the Spanish Council for Scientific Research (CSIC), leading the Laboratory of Ecology and Evolution of Antibiotic Resistance. His research focuses on the molecular bases of antibiotic resistance and the effect of acquiring resistance in the virulence and the overall physiology of bacterial pathogens. He is particularly interested in the use of predictive approaches for studying the emergence of resistance as well as the role that natural (not clinical) ecosystems may have in the origin, evolution, and transmission of antibiotic resistance.

V. F. Lanza graduated in physics, specialized in bioinformatics, and completed doctoral studies in 2015 in the Department of Genetics at the University of Cantabria (Spain), working in bioinformatics of mobile genetic elements. He is a postdoctoral fellow in the Department of Microbiology of the Hospital Ramon y Cajal and Ramón y Cajal Institute for Health Research (IRYCIS); he is currently leading the Bioinformatics Unit at IRYCIS. He has been involved in the bioinformatic study of evolution of antibiotic resistance genes and resistant bacterial populations since 2013. His unit is working on bacterial macro- and microevolution and developing their own software tools, particularly in the field of resistance and plasmid genes in the human microbiome, in part covered by a Research Grant Award of the European Society of Clinical Microbiology and Infectious Diseases.

A. San Millán, D.V.M., Ph.D., is a Group Leader in the National Centre for Biotech-nology in Madrid. He obtained his doctorate on plasmid-mediated antibiotic resistance at the Complutensis University of Madrid in 2010. During his Ph.D. studies, he complemented his training with several stays at the Pasteur Institute in Paris. As a postdoc, he worked for 4 years at the Department of Zoology of the University of Oxford, studying the evolutionary bases of plasmid-mediated antibiotic resistance. In 2016, he started his ERC research group in the Department of Microbiology at Ramon y Cajal University Hospital in Madrid, where he analyzed the evolution of plasmid-mediated antibiotic resistance in patients and the hospital setting. In 2020, Dr. San Millán joined the Spanish National Center for Biotechnology as a Tenured Scientist in Plasmid Biology. Dr. San Millán is interested in understanding the role of plasmids as catalysts of bacterial evolution, with a special focus on the evolution of plasmid-mediated antibiotic resistance in clinical settings.

J. Rodríguez-Beltrán studied biology at the Autonomous University of Madrid and earned his Ph.D. in molecular microbiology at the Institute of Biomedicine of Seville (Spain) in 2015. During his Ph.D. studies, he focused on understanding how recombination and mutation contribute to the development of antibiotic resistance. In 2016, he joined the division of Microbial Biology and Evolution at the Ramón y Cajal Institute for Health Research (IRYCIS) in Madrid as a postdoctoral fellow to study the evolution of plasmid-mediated antibiotic resistance. As a result of his work, he received the Ippen-Ihler Memorial Prize to the best young investigator on plasmid biology. After a research stay at the Pasteur Institute (Paris), he has established his research group at IRYCIS. His research interests focus on understanding the molecular mechanisms that fuel bacterial evolution with the aim of developing new strategies to counter the evolution of antibiotic resistance.

R. Cantón, Ph.D., studied pharmacy at Complutensis University, Madrid (Spain), and obtained his Ph.D. degree in 1994. He was a trainee as a Clinical Microbiology Specialist at the Microbiology Department at the Ramón y Cajal University Hospital at Madrid (Spain), in which he is currently the Head of the Department (since 2011). He is also Associated Professor at Complutensis University. His research activity on antimicrobial resistance, novel techniques on antimicrobial susceptibility testing, and chronic respiratory tract infections (mainly in bronchiectasis and cystic fibrosis) is developed within the Spanish Network for Research in Infectious Diseases (REIPI) and Institute Ramón y Cajal for Health Research (IRYCIS), in which he coordinates the Microbiology, Immunology and Infection Area. He has been Chairman of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and President of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). He has published more than 500 articles in peer-review journals.

J. C. Galán, Pharm.D., Ph.D., studied in Complutensis University, Madrid; a specialist in medical microbiology since 1997, he obtained a doctoral degree in 2002 at this university (genetics of beta-lactamases in anaerobes). He became a staff member in the Microbiology Department of Ramón y Cajal Hospital in 2011, in charge of the area of Virology and Molecular Biology, and Coordinator of the Ramón y Cajal team included in the Center for Network Research in Epidemiology and Public Health (CIBERESP) of the National Institute of Health of Spain. He has been actively working in bacterial hypermutation, phylogeny of bacterial and viral species, and experimental evolution, mainly to reconstruct the evolutionary trajectories of genes involved in antimicrobial resistance. At the present time, his interest is focused on the framework of multiple gene variations involved in the evolution of gene interactions, including antibiotic collateral susceptibility.

T. M. Coque, Ph.D., graduated as a pharmacist and clinical biochemist and received her Ph.D. in medical microbiology from the Complutensis University of Madrid (Spain). A long postdoctoral training (1993–1997) at the University of Texas at Houston (USA) gave her background on molecular epidemiology and genetics of antibiotic resistance. She is a Senior Scientist at the Ramón y Cajal Institute for Health Research in Madrid. Her focus is on studying the ecology and the evolution of opportunistic bacterial pathogens and mobile genetic elements involved in the transmission of antimicrobial resistance for the last 25 years. Advanced -omics applied to the analysis of bacterial populations dynamics is her research interest nowadays. She has published about 170 papers, special issues, and chapters on the field and serves on the editorial boards of several journals. She is/has been a member of international committees (JPIAMR, WHO, EFSA) and evaluation grant panels related to antimicrobial resistance.