ANNUAL REVIEWS

Annual Review of Microbiology

From Petri Dishes to Patients to Populations: Scales and Evolutionary Mechanisms Driving Antibiotic Resistance

Célia Souque, Indra González Ojeda, and Michael Baym

Departments of Biomedical Informatics and Microbiology, Harvard Medical School, Boston, Massachusetts, USA; email: celia.souque@gmail.com, baym@hms.harvard.edu

Annu. Rev. Microbiol. 2024. 78:18.1–18.22

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-041522-102707

Keywords

antibiotic resistance, evolution, mutations, horizontal gene transfer, within-host evolution, transmission

Abstract

Tackling the challenge created by antibiotic resistance requires understanding the mechanisms behind its evolution. Like any evolutionary process, the evolution of antimicrobial resistance (AMR) is driven by the underlying variation in a bacterial population and the selective pressures acting upon it. Importantly, both selection and variation will depend on the scale at which resistance evolution is considered (from evolution within a single patient to the host population level). While laboratory experiments have generated fundamental insights into the mechanisms underlying antibiotic resistance evolution, the technological advances in whole genome sequencing now allow us to probe antibiotic resistance evolution beyond the lab and directly record it in individual patients and host populations. Here we review the evolutionary forces driving antibiotic resistance at each of these scales, highlight gaps in our current understanding of AMR evolution, and discuss future steps toward evolution-guided interventions.

Contents

1. INTRODUCTION

1.1. The Challenge Created by Antibiotic Resistance

The ability to treat bacterial infections safely and efficiently is a cornerstone of modern medicine, and it is currently threatened by the rise of antibiotic resistance. Recent work estimates the annual burden of antimicrobial resistance (AMR) at nearly 5 million associated deaths (104), and resistance to all classes of antibiotics can now be found in pathogenic bacteria (32). Relying on developing new antibiotics has proven to be insufficient to counter resistance, as the pipeline to develop new antibiotics remains clogged (119), and antibiotics in development often exhibit cross-resistance with existing ones (149). Nevertheless, the rise of antibiotic resistance is not inescapable. Deaths from resistant infections in US hospitals fell by 28% between 2013 and 2019, which can be linked to a combination of interventions including infection control and improved antibiotic stewardship (23). However, determining which specific mechanism led to this decline remains challenging. Even simple interventions, such as reducing antibiotic use, often fail to reduce resistance prevalence (46, 145). Similarly, promising strategies to contain resistance evolution in the lab, such as antibiotic cycling (69), struggle to translate into tangible results in clinical trials (153). Therefore, it is crucial to obtain a better understanding of the evolutionary forces shaping antibiotic resistance to determine which interventions will maximize the efficacy of the antibiotics we already have or will develop.

1.2. Evolutionary Biology of Antibiotic Resistance

Antibiotic resistance, defined as the heritable ability of bacteria to survive antibiotic exposure, is an evolution problem. It is a classic example of evolution by natural selection: In the presence of antibiotics, resistant bacteria have a higher chance of survival and replication than susceptible cells.

In this context, antibiotic-resistant cells are said to have a higher evolutionary fitness, defined as the probability of reproductive success of a genotype in a specific environment. Antibiotic resistance evolution is dictated by the combination of variation in resistance traits existing in the population (the initial presence of resistant genes and mutations) with the selective forces determining the fitness of the bacteria bearing these traits (which cells are more likely to survive in a population).

Mobile genetic element: genomic sequence able to move either within or between genomes

1.3. Evolutionary Mechanisms Driving Resistance Emergence

Several evolutionary and ecological mechanisms can generate genetic variation in a bacterial population and therefore give rise to the presence of antibiotic resistance traits. These mechanisms can be classified into four categories (**Figure 1*a***). First is de novo evolution of resistance, such as spontaneous mutations due to errors in DNA replication and repair. In this category, we also include structural rearrangements or intracellular movement of mobile genetic elements to consider all sources of variation that can occur in a clonal population. Second, bacteria can undergo

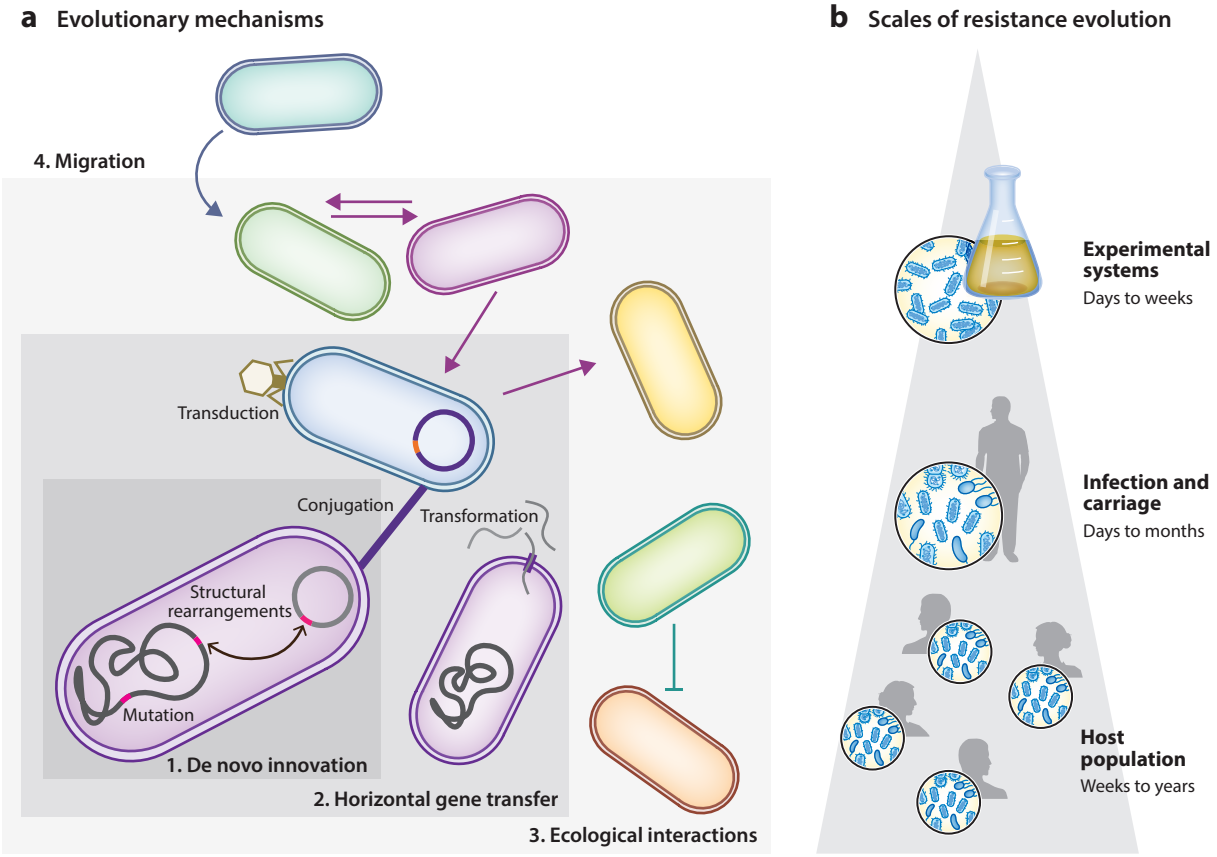

Figure 1

Scales and evolutionary mechanisms driving antibiotic resistance. (*a*) Genetic and phenotypic variations leading to antibiotic resistance occur through a range of mechanisms: de novo innovation (mutation or structural rearrangement happening within an individual bacterial cell), horizontal gene transfer (exchange of genetic material between cells), ecological interactions within members of a bacterial community, and finally migration of resistant bacteria from different environments or sites of the host. (*b*) Antibiotic resistance evolution can occur at many scales (in vitro, in patients, or within host populations) characterized by variations in selective pressures, time frames, and contribution of each evolutionary mechanism.

horizontal gene transfer, where they acquire DNA from unrelated cells, driven by mobile genetic elements or the bacteria themselves. Additionally, the correspondence between resistance phenotype and genotype is not always straightforward. Alongside genetic variation, bacteria often live in polyclonal or polymicrobial communities, which leads to phenotypic variation coming from interactions with other members of the community. We label this third source of variation under the broad umbrella of ecological interactions. Finally, outside of the lab, bacterial populations are often not closed systems, and a substantive amount of variation comes from the migration or translocation of new strains from different environments or parts of the host.

Minimum inhibitory concentration (MIC): lowest concentration of antibiotic preventing visible bacterial growth; used to quantify the resistance phenotype of a bacterial population

Persistence: ability of a subpopulation of bacteria to survive antibiotic exposure; these survivors are called persister cells

Tolerance: ability of a bacterial population to transiently survive exposure to lethal doses of antibiotics

1.4. Scales of Evolution

Over long timescales, these mechanisms are not independent from each other: Genes transferred through horizontal transfer originally arise through mutations, and novel bacterial strains or species arise from variation acquired through mutation or horizontal gene transfer. However, these mechanisms can be distinct at the scales we focus on to counter antibiotic resistance: evolution within a patient (carriage or infection) or evolution at the level of the (human) host population (**Figure 1*b***). Each of these scales is defined by specific selection pressures and opportunities for variation, therefore altering the contributions of each evolutionary mechanism to antibiotic resistance.

The study of the evolutionary mechanisms of antibiotic resistance has long been driven by experimental evolution and other experimental approaches in model systems, but the development and popularization of whole genome sequencing in recent years have opened a new door to investigate resistance evolution directly as it happens in patients and populations. Starting from experimental work (Section 2) before going to patients (Section 3) and populations (Section 4), we review our current understanding of the evolutionary mechanisms driving antibiotic resistance at each of these scales and how it may inform evolutionary-guided interventions to contain AMR. This review does not claim to be exhaustive, but it aims to provide a framework to summarize and conceptualize our current understanding of resistance evolution. In the interest of space, we primarily focus on antibiotic resistance defined as a heritable trait linked with an increase in antibiotic minimum inhibitory concentration (MIC), in opposition to persistence and tolerance, for which we point the reader toward additional reviews (18, 50).

2. EVOLUTION IN EXPERIMENTAL SYSTEMS

The study of antibiotic resistance evolution in the lab is nearly as old as the discovery of antibiotics themselves, with pioneering work on salvarsan resistance emergence in the 1910s (2). In this section, we review the current understanding obtained from experimental studies of the role of mutations, horizontal gene transfer, and community interactions in the evolution of antibiotic resistance.

2.1. Evolution Through Mutation and De Novo Innovation

Experimental evolution studies have historically focused on the de novo innovations that arise in a clonal population, in part due to ease of study. In this context, antibiotic resistance can emerge easily, but the evolutionary success of these mutants depends on the fine balance between the fitness benefit they provide and the cost their bacterial host incurs.

2.1.1. Emergence of resistance from mutations is frequent. Resistance to nearly all antibiotic classes can be readily evolved in vitro through mutations (82). The mutation rate, defined as the frequency at which detectable resistant mutants arise in a bacterial population, varies for each

bacterial species and antibiotic combination, spanning more than six orders of magnitude (67). It heavily depends on the antibiotic concentration considered: Mutations that provide high levels of resistance are usually rare, while mutations that provide low levels of resistance are much more frequent (42, 172).

The mutation rate is also impacted by bacteria physiology, such as induction of the SOS response or in response to starvation (98). As such, antibiotics themselves can act on mutation rates. For example, norfloxacin increases mutation rate by an order of magnitude in *Escherichia coli* (92). On the extreme is the so-called hypermutator phenotype, where mutations in the DNA repair pathway can lead to up to a 10- to 1,000-fold increase in mutation rate (54). Polyploidy can also accelerate the evolution of resistance by increasing the number of targets for mutations: Novel resistant mutations have been shown to occur more frequently when a gene is on a multicopy plasmid versus on the chromosome (127). Mutations on polyploid targets may, however, be subject to genetic dominance, where dominant sensitive alleles can block the impact of recessive resistant mutations (125, 144).

2.1.2. Resistance mutations incur a fitness cost. Given the mutation rate (usually above 10^{-10} per nucleotide per generation) and the size of bacterial populations found in infections ($\sim 10^{10}$ CFU), evolution of resistance by a single mutation is not generally constrained by mutational supply (67). Thus, the probability of a mutation persisting in the population will be a function of the selective advantage it provides to its host. The selective advantage obtained from a mutation depends on both the benefits (e.g., level of resistance) and the fitness cost it creates in the absence of antibiotic, as resistance mutations often occur in essential pathways and directly impair their function (101). Antibiotic resistance mutations are often pleiotropic and may also generate indirect costs, for example by restricting niche breadth (62). Another example of pleiotropy is collateral sensitivity, where resistance to one antibiotic leads to increased sensitivity to another (146). For example, modifications of the cell membrane reducing the uptake of aminoglycosides impede efflux pumps, leading to increased susceptibility to several other antibiotic classes (82).

Even when antibiotic resistance mutations are costly, these costs can be mitigated, and even eliminated, by further compensatory mutations (10), which can stabilize resistant mutations in a population even in the absence of antibiotic selection. Compensatory mutations are an example of a broader phenomenon named epistasis, under which the cost and impact of resistant mutations depend on the genomic background in which they occur (155), which in turn impacts the trajectory of resistance evolution (96).

2.1.3. De novo resistance evolution is not limited to mutations. Finally, resistance evolution in clonal populations can also occur through structural rearrangements (insertions, duplications, etc.), at even higher rates than point mutations (5). These rearrangements are facilitated by mobile genetic elements such as integrons and insertion sequences (22, 136). Duplications in particular can lead to unstable amplification and overexpression of resistance genes and generate transient but heritable resistance (109).

2.2. Evolution Through Horizontal Gene Transfer

Horizontal gene transfer is the exchange of genetic material between potentially unrelated organisms. It provides bacteria access to extensive genetic novelty but also affects evolutionary dynamics at the gene level.

2.2.1. Horizontal gene transfer modifies the evolutionary dynamics of resistance. The contribution of horizontal gene transfer to antibiotic resistance is enormous, as it allows the

Pleiotropy: phenomenon where a single mutation impacts several apparently unrelated phenotypic traits

Epistasis: phenomenon where the effect of a mutation is impacted by the presence of other genes or mutations

Integron: genetic platform allowing the capture, expression, and shuffling of mobile gene cassettes often encoding for antibiotic resistance

movement of genes across bacterial species and ecosystems as well as the acquisition of multidrug resistance in a single event, due to the frequent colocalization of resistance determinants on the same mobile genetic element (113).

Horizontal gene transfer is a stabilizing force for the presence of resistance in bacterial populations. The cost of resistance from acquired resistance genes is usually less than the cost of resistance from mutations, potentially due to ongoing adaptation of the resistance genes as they move between hosts (156). The genetic linkage between resistance genes on mobile genetic elements also facilitates coselection of resistance, where selection for a single resistance gene leads to maintenance of the entire multidrug resistance island. Additionally, in the presence of horizontal gene transfer, the persistence of a resistance gene becomes the product of its ability to transmit both vertically (from mother to daughter cells, similarly to chromosomal mutations) and horizontally (between unrelated cells). This phenomenon can lead to the persistence of resistance even in the absence of selection if horizontal gene transfer rates are high enough (93, 139) and is often reinforced by the presence of addiction systems preventing the loss of mobile genetic elements (167).

2.2.2. Horizontal gene transfer occurs through several mechanisms. Mechanistically, horizontal gene transfer can be mediated by mobile genetic elements (conjugation and transduction) or promoted by the bacterial host itself (natural transformation). Each of these mechanisms is characterized by varying efficiency, host range, and genetic cargo capacity.

Conjugation, especially via conjugative plasmids, is the best understood mechanism of horizontal gene transfer, in which genetic material is exchanged from a donor to recipient cell through a conjugative pilus. Plasmid conjugation rates are extremely variable but can exceed mutation rate by several orders of magnitude (3). Conjugation rates have been shown to depend on donor-recipient relatedness, presence of other plasmids, and abiotic factors, such as planktonic or solid environments (3, 15). Similarly to resistance mutations, resistance plasmids often generate a fitness cost for their host (128). The molecular mechanisms driving the cost of plasmid carriage remain poorly understood but have been shown to be highly host dependent (43).

The evolutionary dynamics of transduction and transformation are by comparison much less studied, but both can be upregulated in response to antibiotics (8, 120).

Bacteriophages rarely carry resistance determinants as part of their genomes [except for the recently described plasmid-phages (116)], but they can transfer resistance genes by mistakenly packaging DNA from their host (either chromosomal or plasmid) alongside their own in a process called transduction. Bacteriophages have a narrower host range than plasmids, and the rate of transduction is thought to be much smaller than conjugation (106), but recent results point toward the potential for much higher transduction rates (25).

Finally, natural transformation, the ability of bacteria to take up and integrate DNA from their environment, is an ancient trait that predates the separation between Gram-negative and Gram-positive bacteria (73). It is tightly regulated, making its study in the lab challenging (14). Natural transformation has been shown to allow the transfer of both resistance alleles (164) and entire mobile genetic elements, such as integrons and transposons (41, 152).

2.3. Impact of Ecological Interactions and Complex Environments on Resistance Evolution

Bacteria in the environment do not live in the clonal, planktonic cultures that are most often studied in experimental settings but instead live in genetically diverse communities and complex environments that can impact both variation and selection for antibiotic resistance (17).

2.3.1. Community composition alters variation and selection for resistance. The simplest example of the impact of community composition on drug resistance is the effect of bacterial density on antibiotic inhibition (64), with drugs showing decreased or increased inhibition at high density (76). At a fundamental level, the increase in standing genetic variation found in a polymicrobial versus clonal population can accelerate adaptation (7). Diversity in hosts may also stabilize mobile genetic elements by generating source-sink dynamics between species (60).

Interactions between members of a community can modify the impact of selection and either hamper or favor resistant strains. For example, Klümper et al. (80) embedded isogenic strains of *E. coli* with and without a resistance gene in a pig fecal community and showed the resident community decreased the strength of selection for resistance by either increasing the cost of the resistance gene or protecting the susceptible strain. On the other hand, the killing of a sensitive strain by antibiotic treatment can free up nutrients for the resistant strain, giving it a competitive advantage against the rest of the community (competitive release) (110). Additionally, several antibiotic resistance mechanisms are cooperative, where resistant bacteria can protect the rest of the population (154), for example through the production of antibiotic-degrading enzymes, which will also protect nonproducer cells (114). These social interactions between strains can lead to complex dynamics in the prevalence of resistance, such as cheating, where sensitive (cheater) cells do not incur the fitness cost of resistance but benefit from the protection provided by the resistant cells, limiting the spread of resistant genes in the population (170).

2.3.2. Complex environments impact resistance evolution. The physical nature of the environment can heavily shape the evolution of antibiotic resistance. Bacteria often live in biofilms, complex matrices of secreted polymers. Biofilms are characterized by their heterogeneity: The matrix itself can impede the diffusion of both antibiotics and nutrients, leading different parts of the population to experience varying selection pressures (138). As such, biofilms often contain bacterial subpopulations in different physiological states (40). The spatial structure of biofilms also restricts competition between mutants, lessening the impact of selection (129).

The environment may impact both fitness costs and rates of horizontal gene transfer. While a meta-analysis found no statistical difference between the cost of resistance in vitro and in mice (156), several studies have shown considerable variation in the fitness and evolution of resistance between bacteria evolved in urine or synthetic sputum versus traditional rich media (66, 81). Similarly, rates of horizontal gene transfer can vary between in vitro and in vivo systems. For example, many plasmids conjugate in vitro but fail to conjugate in mice (107); on the contrary, high rates of transduction in piglets have been impossible to replicate in vitro (100).

3. EVOLUTION WITHIN INDIVIDUAL PATIENTS

The developments in whole genome sequencing have made it possible to track antibiotic resistance dynamics during infection and colonization (105). Evolution of resistance has been witnessed during both chronic and acute infection in response to treatment, as well as from bystander selection in microbiomes under antibiotic exposure. In this section, we review the recent discoveries from genomic studies on the mechanisms driving both variation and selection for resistance within patients.

3.1. Mechanisms Driving Genetic and Phenotypic Variation of Resistance in Patients

In patients, types of infections differ in their initial diversity and in opportunities to acquire genetic variation over time.

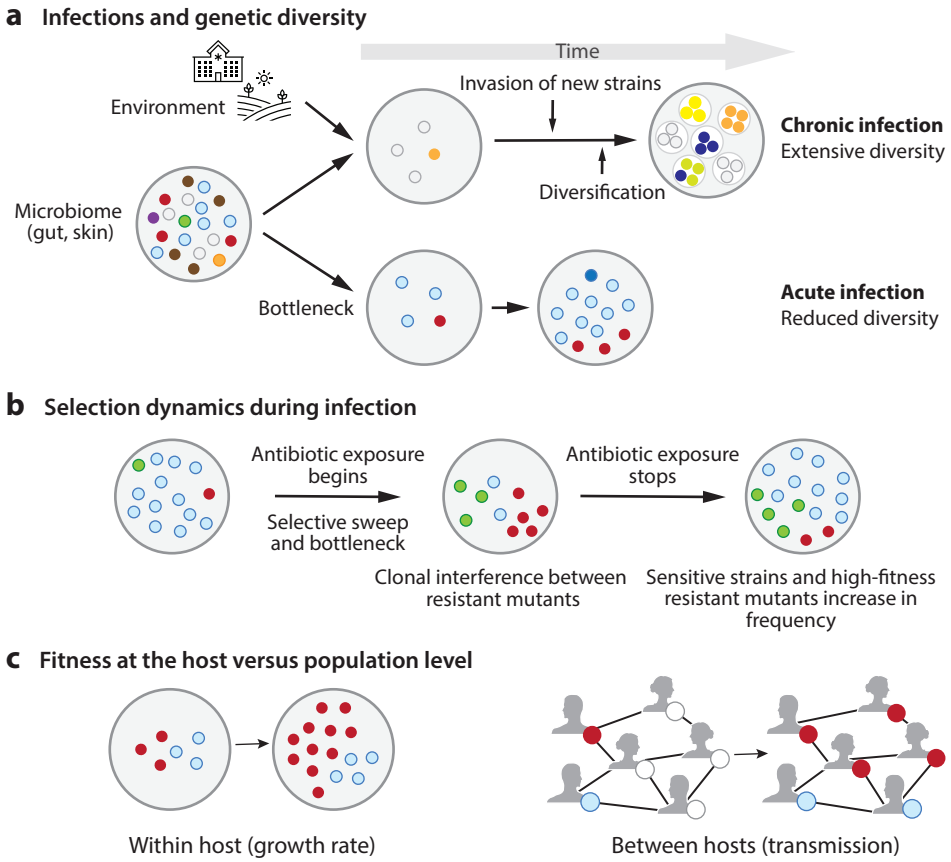

Figure 2

Evolutionary dynamics within patients and populations. (*a*) Genetic diversity in infections fluctuates based on duration and type of infection. After an initial bottleneck that heavily constrains genetic diversity, novel diversity can arise through several mechanisms. This process is facilitated in chronic infection where environmental heterogeneity helps generate diverse niches. (*b*) Competition drives the rise and fall of resistant genotypes during infection. Antibiotic exposure impacts bacterial populations by both reducing the population size (bottleneck) and creating selective sweeps for resistant bacteria (full circles). However, these selective sweeps rarely lead to fixation due to competition between resistant mutants (clonal interference). Finally, once the antibiotic selective pressure is removed, resistant bacteria may be outcompeted by sensitive or less resistant clones with higher fitness. (*c*) Evolutionary success differs based on the scale considered. At the population level, the success of a bacterial strain is defined by its ability to replicate within a host (replicative fitness) but also its ability to move between and colonize new hosts (transmission fitness).

3.1.1. Infection types differ in initial genetic variation. Chronic infections, with their large population size and long duration, often contain extensive genetic and phenotypic diversity (38) (**Figure 2*a***). One of the most studied systems of chronic infection is the cystic fibrosis (CF) lung, characterized by *Pseudomonas aeruginosa* or *Staphylococcus aureus* infections spanning decades (49). Initial colonization of the lung is followed by extensive diversification in both genotypes and resistance profiles, driven by the heterogeneity of the CF environment (36, 97). Enhanced diversity is also frequent in other chronic infections, such as *Helicobacter pylori*, where divergent lineages with diverse resistance profiles have been identified in different part of the stomach (132), and

in tuberculosis with genetic variation coming from both mixed infections and de novo variation, with sublineages coexisting for years (88).

By comparison, acute infections start from a recent bottleneck, frequently leading to a nearly clonal initial population (26, 159). However, recent studies have highlighted an underappreciated genetic diversity at the onset of acute infections, coming from either mixed infections or genetic diversity within the same clone (86) and correlating with previous antibiotic exposure (26). This initial diversity can directly impact the likelihood of resistance emergence. For example, a study of acute *P. aeruginosa* lung infections showed antibiotic resistance evolved faster in polyclonal than single clone infections due to selection on preexisting variation (37).

As infections are frequently seeded from the host microbiome (103, 169), carriage of resistant strains is a strong risk factor for future resistant infections (121). Microbiomes, in locations such as the skin or the gut, are complex environments acting as a reservoir of both resistance strains and genes (65, 135). After colonization by a resistant strain, rapid diversification can often lead to coexistence of both resistant and sensitive variants. Some examples include the rapid loss of *mecA*, and structural variations in the SCC*mec* cassette have been shown in *Staphylococcus epidermidis* as it colonizes newborns (30), while a sensitive version of the pOXA48 plasmid was found to emerge rapidly and coexist in the gut of a patient colonized by carbapenem-resistant *Klebsiella pneumoniae* (34).

3.1.2. In-patient evolution of resistance can happen through mutations and structural rearrangements. Alongside initial variation in resistance, in situ emergence of resistance during chronic infection has been repeatedly observed, especially through mutations. *Mycobacterium tuberculosis* infections are a typical example where in situ resistance evolution is purely driven by mutations: Evolution of an infection from fully susceptible to pan resistant has been observed through successive mutational steps (44). Resistance through mutations can also occur in the short time frame of an acute infection (26, 158), with a *P. aeruginosa* double mutant appearing in less than 4 days under ciprofloxacin treatment, leading to treatment failure (83).

Additionally, hypermutator strains with enhanced mutation rate have been repeatedly identified in infections, especially in CF (111) and urinary tract infections (UTIs) (35). In UTIs, it was found that hypermutators can contribute up to 50% of the overall genetic diversity (19). Evolution of resistance through mutation in patients is not limited to chromosomal targets but has also been observed on plasmids through mutations increasing plasmid copy number (34, 159). Finally, intracellular rearrangements driven by mobile genetic elements have also been reported to contribute to emergence of resistance, through rampant movement of insertion sequences in *P. aeruginosa* disrupting antibiotic import (133) as well as integron cassette shuffling leading to increased expression of a beta-lactamase (63).

3.1.3. Horizontal gene transfer drives evolution of resistance in the microbiome. Plasmid-mediated resistance is the main driver of resistance in *Enterobacteriaceae* pathogens, with the gut microbiome acting as a hotspot for the spread of resistance plasmids through conjugation (75, 85). New techniques such as Hi-C are enabling the tracking and quantification of plasmid transfer directly from microbiome samples (78). Kent et al. (78) identified unique networks of horizontal gene transfer within the gut of various patients, with a constant basal level of horizontal gene transfer even in the absence of antibiotic treatment (see also 58). Otherwise, evidence of the contribution of conjugation to resistance evolution during infection remains sparse: In CF, *P. aeruginosa* was found more likely to lose DNA than acquire new elements, (123) and more plasmid loss than gain was identified during a recurrent UTI (52).

The overall contribution of transduction to the spread of resistance remains to be determined. Potential for transduction-mediated transfer of resistance has been demonstrated on the skin, with

rampant transduction of *S. aureus* on the skin of piglets (100). Similarly, phages isolated from the mice gut post antibiotic treatment increase resistance prevalence when transferred to a naïve population (102). However, while studies report identification of phages containing antibiotic resistance genes from feces (122) and CF lungs (48), it has been shown that identification of resistance genes from viromes is prone to false positives (45).

Finally, examples of the impact of natural transformation on resistance evolution within infection are limited, but extensive homologous recombination has been observed in *H. pylori* during infection (21), and important pathogens such as *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Neisseria gonorrhoeae* are known to be highly competent (14).

3.1.4. Infection microenvironment alters resistance phenotype. Resistance phenotypes within an infection do not always correlate with resistance measured in standard microbroth assays, especially as many infections present as biofilms. For example, successful treatment in a pig model of implant infection by *S. aureus* required more than 100 times the planktonic MIC (71), and *E. coli* was found to survive in the bladder at concentrations higher than the MIC (11). Mismatch between antibiotic susceptibility in vitro and treatment success is of particular concern in CF (134), reinforced by the complex interactions between the coexisting bacterial species (16).

3.1.5. Migration and translocation contribute to genetic diversity during infection. In comparison to lab experiments, infections are often not closed systems. Migrations of bacteria within the infection site have been observed in both *H. pylori* and *M. tuberculosis* (1, 88). By comparison, bacteria in CF infections present little to no mixing between parts of the lung (74). Movement of bacteria between body sites is also common, and recurrent translocation from the gut to the bladder of the same *E. coli* strain is a frequent source of recurrent UTIs (148). Migration can provide genetic diversity even during the short time frame of an acute infection, with gut to lung transmission detected during a lung infection (159). Finally, genetic diversity can be acquired by invasion of new strains from the environment, as shown by the acquisition of resistant strains in the gut microbiome during travel (165) or strain replacement by epidemic strains of *P. aeruginosa* observed in CF, which was shown to provide more genetic diversity than in situ diversification (161). Similarly, a large-scale study of UTI reoccurrence showed that strain replacement, more than mutation or horizontal gene transfer, was the main driver of resistance emergence in recurrent UTIs and was strongly impacted by antibiotic exposure history (141).

3.2. Dynamics of Selection for Antibiotic Resistance in Patients

Over the course of an infection, bacterial populations experience fluctuating selection pressures from different sources, such as antibiotic use, competition with other bacteria, and the host's immune system.

3.2.1. Antibiotic treatment generates rapid evolution of bacterial populations. Antibiotic use during infection is frequently linked with rapid selective sweeps of resistant mutants. Using targeted deep amplicon sequencing of select resistance mutations, Chung et al. (26) showed in acute *P. aeruginosa* lung infections that both de novo and preexisting mutations can increase in frequency within days in response to antibiotic treatment. Similarly, exposure to antibiotics is often associated with an increase in prevalence of resistance mutations and resistance genes in the gut microbiome (33, 162, 168). Alongside increasing the relative frequency of resistance, antibiotic treatment often acts as a bottleneck reducing the population diversity, even in the presence of an already resistant population (1, 151).

As mentioned previously, the selection pressure created by antibiotic treatment is highly dependent on the infection environment: Treatment suppressing *P. aeruginosa* in the lung did

not impact the same bacterial population found in the gut of a patient (158). Likewise, CF communities have been found to be very robust to the high dose of antibiotics used during exacerbations (29, 51), potentially due to the complex community and the high prevalence of resistance genes.

3.2.2. Competition drives rise and fall of resistant genotypes. The selective sweeps in response to antibiotic exposure seldom lead to complete fixation of resistance (44, 143) in part due to competition between resistant mutants, which slows fixation (clonal interference) (87). Sensitive subpopulations can also survive antibiotic treatment (1, 159). This surviving diversity will impact the resistance dynamics once the selective pressure for resistance subsides. High-resistance, low-fitness mutants can be replaced by either higher fitness, lower resistance mutants (158) or sensitive strains (159). These dynamics can be very quick as well as environment dependent: Chung et al. (26) found that resistant mutations for antibiotics not currently administrated went extinct within days, and Wheatley et al. (159) found that a meropenem-resistant mutant was quickly outcompeted by a sensitive lineage in the gut but remained stable in the lung. Interestingly, how quickly resistance recedes in a bacterial community can be impacted by its genomic localization (e.g., chromosomal mutations versus mobile genetic elements), with chromosomal mutations decreasing in prevalence in the gut more quickly than mobilizable genes, potentially due to horizontal gene transfer (168).

3.2.3. The immune system shapes resistance evolution. The immune system is an important selection pressure in the host environment (38), which can also indirectly alter resistance evolution. Host immunity can strongly impact bacterial load, reducing it by a factor of 10 before antibiotic treatment even starts (158), and can be the main driver leading to the resolution of an infection (158). Serial passaging of *A. baumannii* in immunodeficient and immunocompetent mice showed that resistance evolution is constrained by the immune system, with resistant mutants arising more frequently in immunosuppressed mice (68). On the other hand, inflammation has been shown to boost horizontal gene transfer in the gut. *Salmonella*-inflicted enteropathy leads to bloom of *Enterobacteriaceae* and reduces the prevalence of commensal bacteria in the gut, which otherwise physically block conjugation between *Enterobacteriaceae* (137).

Antibiotics can also act synergistically with the immune system: Beta-lactams have been shown to sensitize methicillin-resistant *S. aureus* (MRSA) to host immune peptides as well as human-made antimicrobial peptides, such as colistin and daptomycin (126). However, the therapeutic use of antimicrobial peptides can be problematic, as resistance to antimicrobial peptides can also lead to cross-resistance to host immune peptides (59). This can result in increased virulence, as bacteria resistant to antimicrobial peptides are then able to evade the immune system more efficiently (70).

4. EVOLUTION AT THE HOST POPULATION LEVEL

While de novo emergence of resistance within patients impacts clinical outcome, the main contributor to antibiotic resistance burden remains infections by already resistant pathogens (12, 77). As such, predicting the dynamics of resistance at the population scale requires understanding both emergence and transmission of resistant bacteria.

4.1. Epidemic Clones and Successful Mobile Genetic Elements Drive Antibiotic Resistance at the Population Level

Within populations of pathogens, certain high-risk subpopulations disproportionately contribute to the burden of antibiotic resistance.

4.1.1. High-risk epidemic clones are major contributors of resistant infections. The use of whole genome sequencing in epidemiology has revealed that many resistant infections are driven by the clonal expansion of a few high-risk clones in both Gram-positive and Gram-negative bacteria (160, 163). A recent survey found that 70% of carbapenem-resistant *K. pneumoniae* infections came from only four clonal lineages (31), while *E. coli* ST131 made on average 40% of all extended-spectrum beta-lactamases (ESBL)-producing *E. coli* isolates in Europe (108). These high-risk clones often combine high fitness and transmission ability with extensive levels of antibiotic resistance. *E. coli* ST131 has been shown to carry ESBL plasmids at little to no fitness cost (131) and can adhere to host epithelial cells (130) as well as displace commensal *E. coli* even in the absence of antibiotic treatment (27). Similarly, *S. aureus* ST8:USA300 is resistant to methicillin through near cost-free carriage of a SCC*mec* element and contains virulence factors that enhance its carriage and competitive ability in vivo (39, 150).

4.1.2. High-risk clones arise through a combination of evolutionary mechanisms. Both mutations and horizontal gene transfer have been shown to contribute to the emergence of high-risk clones. Acquisition of resistance to fluoroquinolones through mutations of *gyrA*, *gyrB*, and *parC* has been identified several times in epidemic clones (53). The acquisition of ciprofloxacin resistance soon after its introduction in the clinic is thought to be one of the main drivers of ST131 success (9). Similarly, horizontal gene transfer has been a key factor in the rise of MRSA clones: Acquisition of the mobile methicillin resistance element SCC*mec* by horizontal gene transfer is estimated to have occurred at least 20 times within *S. aureus* phylogeny (124). Plasmid-borne resistance determinants are also associated with many high-risk clones, such as CTX-M beta-lactamases in *E. coli* ST131 (140) and the carbapenemase KPC in *K. pneumoniae* ST258 (99).

These high-risk clones are not genetically static but are instead often characterized by a high variability in the resistance determinants they carry. For example, *P. aeruginosa* ST235 has been identified with more than 100 different resistance elements (112) and *E. coli* ST131 with different variants of CTX-M (140). Finally, the dominance of specific clones is very dynamic, with now declining prevalence of *S. aureus* USA300 (118) and replacement of *E. coli* ST131 by ST1193 in certain regions (117).

4.1.3. Mobile genetic elements can spread resistance globally. Alongside epidemic bacterial clones, the spread of resistance across the globe has also been driven by successful mobile genetic elements. A prime example of this is the class 1 mobile integron whose spread was driven by the use of hospital disinfectants and sulfonamides in the 1930s (55). They are now found in between 40% to 70% of Gram-negative bacteria, usually carrying 1 to 5 resistance genes (55). Integron presence is correlated with anthropogenic antibiotic use to such an extent that the prevalence of the integron integrase *IntI1* has been suggested as a marker for antibiotic contamination in soil and water bodies (56). More recently, the mobilization in the mid-2000s in China of the colistin resistance gene *mcr1* on an ISApl1 transposon and IncI2 and IncX4 plasmids was then followed by its rapid global dissemination (157). The contribution of mobile genetic elements to the spread of resistance can also be seen at a local scale, with recent increased awareness of plasmid outbreaks occurring in hospitals (47, 115).

4.2. Selection and Transmission of Resistance Within Populations

The spread of bacteria within a host population is characterized by an additional layer of selection, whereby resistant and sensitive strains compete not only to survive within a host but also to transmit between hosts.

4.2.1. Within- versus between-host evolutionary success. Transmission plays a crucial role in the spread of resistance at the population level, as highlighted by the contribution of epidemic clones and successful mobile genetic elements to resistance prevalence. At the population level, the success of a bacterial strain becomes defined by its ability to replicate within a host (replicative fitness) but also its ability to move between and colonize new hosts (transmission fitness) (89) (**Figure 2*c***). The transmission fitness of a new resistant mutant will be highly situational: High-fitness mutants arising in a part of the body that does not permit onward transmission (such as the blood) can be considered an evolutionary dead-end [adapt-and-die versus adapt-and-live mutations (28)] with a transmission fitness of zero. As such, not all resistance arising within patients will contribute similarly to the prevalence of resistance at the population level.

For resistance that arises in a transmissible background and environment, how much resistance will impact transmission remains an open question. We know that resistance can impair colonization and transmission of *Campylobacter jejuni* in comparison to a sensitive strain (94), and that increased transmissibility is associated with compensatory mutations in rifampicin-resistant tuberculosis (57), but it is unclear if resistance is always linked with reduced transmission.

4.2.2. Transmission dynamics shape resistance prevalence. Additionally, transmission generates an additional layer of complexity to the dynamics of resistance. At the population level, long-term coexistence between resistant and sensitive bacterial populations has been observed in several species [*E. coli*, *S. pneumoniae* (12, 84)]. This observation is puzzling, as simple ecological models suggest resistance should sweep through a population as long as antibiotic use leads to increased fitness of the resistant strain (12). It has therefore been suggested that the prevalence of resistance may be constrained by heterogeneous transmission rates within the host population (13) or linkage with other traits under frequency-dependent selection such as duration of carriage (84). The latter is another great example of how transmission dynamics can impact resistance evolution, as longer duration of carriage leads to higher chance of exposure to antibiotics, increasing the fitness of resistant strains (84). Finally antibiotic use itself can impact transmission of resistant bacteria by disturbing preexisting bacterial communities, facilitating colonization by resistant organisms that may not otherwise be able to invade (90).

4.2.3. Transmission and selection within and between environments. Selection pressure and opportunities for transmission differ based on the environment, visible in the separation between hospital- and community-acquired strains in species such as *S. aureus* and *K. pneumoniae*. In the community, exposure to antibiotics is sparse at the level of an individual host, and it has been shown that for many bacterial species, most of the antibiotic exposure they experience is the result of treatment for a condition they did not cause (147). While inpatient antibiotic usage represents only 10% of overall antibiotic consumption (4), the high rate of antibiotic use and the increased density of susceptible hosts make hospitals hotspots for antibiotic resistance evolution (4), with hospital-associated strains usually displaying more antibiotic resistance than their community counterparts (24, 166). In hospitals, transmission of resistant bacteria is enhanced by colonization of the built environment, notably through biofilms in sink traps acting as reservoirs of resistance (142).

Selection for antibiotic resistance may happen not only in human hosts but also in agriculture settings or in any environment polluted by antibiotic-containing effluents (61). As mobile genetic elements allow the transfer of resistance genes across bacterial species and habitat boundaries, there is a growing concern that the use of antibiotics outside of human health promotes transmission of resistance genes back into human populations, requiring a One Health approach to antibiotic resistance (61). An example of this danger is the emergence of *mcr1* from agricultural settings in China, following decades of colistin use in agriculture (now banned) (91, 157).

However, how frequently resistance genes and mobile genetic elements move between agricultural and clinical environments remains an open question, as several studies have found little overlap in the mobile genetic elements content of agricultural and clinical bacterial populations (20, 95).

5. PERSPECTIVE: TOWARD EVOLUTION-GUIDED INTERVENTIONS

Experimental work has generated not only knowledge about the fundamental mechanisms of resistance evolution but also a wealth of potential approaches to act at the level of both variation and selection for resistance, ranging from leveraging collateral sensitivity through antibiotic cycling (79) to developing antievolvability drugs targeting stress-induced mutagenesis (171). But which of these ideas and strategies will make a tangible impact on resistance evolution is much harder to determine and is limited by the many blind spots that remain in our understanding of how resistance behaves within hosts and populations. For example, while cycling and combination of antibiotics have shown potential with mutation-based resistance, their relative efficacy will also depend on the starting genetic variation (72) and the potential for coselection of mobile genetic elements and influx of mutants within a population (6), at both patient and hospital levels. New strategies that consider future evolution will require first a more detailed understanding of how resistance evolves in natural environments beyond the laboratory.

The clinical use of antibiotics has effectively provided us with 80 years of experimental evolution, and we now have the technology to observe the results and begin to answer these questions. The rise of affordable and high-throughput whole genome sequencing now allows us to study the dynamics of antibiotic resistance as they occur, both within patients and populations. While we have historically focused on sequencing resistant bacteria, we now have the capacity, through large-scale sampling of both resistant and sensitive bacteria, to understand when resistance evolves as much as when it does not. Understanding what dynamics already constrain the evolution of resistance in populations will allow us to design future interventions that account for evolution and are not so readily countered by it.

FUTURE ISSUES

1. We need improved experimental systems that reproduce the environment and the diversity found in infections, as well as experimental systems that investigate the parameters affecting transmission.
2. A better understanding of the evolutionary parameters found in infections, such as population size, diversity, migration, bottlenecks, and selection pressures, will improve the predictive power of experimental systems.
3. To understand resistance evolution at the patient level, we need to look beyond individual patients by characterizing and quantifying the risk factors of each evolutionary mechanism across cohorts.
4. At the population level, a better understanding of the biology driving the evolution of high-risk clones will allow us to forecast future threats, guiding surveillance.
5. Beyond the discovery of new antibiotics, the identification of additional biological mechanisms that hinder the spread of resistance at the population level, such as phage predation or competition between mobile genetic elements, will enable the development of evolutionarily robust strategies to counter antibiotic resistance.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the members of the Baym lab for fruitful discussions and feedback during the writing of this review. This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health (grants R35GM133700 and T32GM008313) and the Pew Charitable Trusts. I.G.O. received support from the National Science Foundation Graduate Research Fellowship Program.

LITERATURE CITED

1. Ailloud F, Didelot X, Woltemate S, Pfaffinger G, Overmann J, et al. 2019. Within-host evolution of *Helicobacter pylori* shaped by niche-specific adaptation, intragastric migrations and selective sweeps. *Nat. Commun.* 10(1):2273
2. Akatsu S, Noguchi H. 1917. The drug-fastness of spirochetes to arsenic, mercurial, and iodide compounds in vitro. *J. Exp. Med.* 25(3):349–62
3. Alderliesten JB, Duxbury SJN, Zwart MP, de Visser JAGM, Stegeman A, Fischer EAJ. 2020. Effect of donor-recipient relatedness on the plasmid conjugation frequency: a meta-analysis. *BMC Microbiol.* 20(1):135
4. Andersson DI, Balaban NQ, Baquero F, Courvalin P, Glaser P, et al. 2020. Antibiotic resistance: turning evolutionary principles into clinical reality. *FEMS Microbiol. Rev.* 44(2):171–88
5. Andersson DI, Hughes D. 2009. Gene amplification and adaptive evolution in bacteria. *Annu. Rev. Genet.* 43:167–95
6. Angst DC, Tepekule B, Sun L, Bogos B, Bonhoeffer S. 2021. Comparing treatment strategies to reduce antibiotic resistance in an in vitro epidemiological setting. *PNAS* 118(13):e2023467118
7. Barrett RDH, Schluter D. 2008. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23(1):38–44
8. Bearson BL, Brunelle BW. 2015. Fluoroquinolone induction of phage-mediated gene transfer in multidrug-resistant *Salmonella*. *Int. J. Antimicrob. Agents* 46(2):201–4
9. Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM, Khanh Nhu NT, Roberts LW, et al. 2016. Sequential acquisition of virulence and fluoroquinolone resistance has shaped the evolution of *Escherichia coli* ST131. *mBio* 7(2):e00347-16
10. 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(5457):1479–82
11. Blango MG, Mulvey MA. 2010. Persistence of uropathogenic *Escherichia coli* in the face of multiple antibiotics. *Antimicrob. Agents Chemother.* 54(5):1855–63
12. Blanquart F. 2019. Evolutionary epidemiology models to predict the dynamics of antibiotic resistance. *Evol. Appl.* 12(3):365–83
13. Blanquart F, Lehtinen S, Lipsitch M, Fraser C. 2018. The evolution of antibiotic resistance in a structured host population. *J. R. Soc. Interface* 15(143):20180040
14. Blokesch M. 2016. Natural competence for transformation. *Curr. Biol.* 26(21):R1126–30
15. Bottery MJ. 2022. Ecological dynamics of plasmid transfer and persistence in microbial communities. *Curr. Opin. Microbiol.* 68:102152
16. Bottery MJ, Matthews JL, Wood AJ, Johansen HK, Pitchford JW, Friman V-P. 2022. Inter-species interactions alter antibiotic efficacy in bacterial communities. *ISME J.* 16(3):812–21
17. Bottery MJ, Pitchford JW, Friman V-P. 2021. Ecology and evolution of antimicrobial resistance in bacterial communities. *ISME J.* 15(4):939–48
18. Brauner A, Fridman O, Gefen O, Balaban NQ. 2016. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat. Rev. Microbiol.* 14(5):320–30

19. Bridier-Nahmias A, Launay A, Bleibtreu A, Magnan M, Walewski V, et al. 2021. *Escherichia coli* genomic diversity within extraintestinal acute infections argues for adaptive evolution at play. *mSphere* 6(1):e01176-20
20. Calland JK, Haukka K, Kpordze SW, Brusah A, Corbella M, et al. 2023. Population structure and antimicrobial resistance among *Klebsiella* isolates sampled from human, animal, and environmental sources in Ghana: a cross-sectional genomic One Health study. *Lancet Microbe* 4(11):e943–52
21. Cao Q, Didelot X, Wu Z, Li Z, He L, et al. 2015. Progressive genomic convergence of two *Helicobacter pylori* strains during mixed infection of a patient with chronic gastritis. *Gut* 64(4):554–61
22. Card KJ, Thomas MD, Graves JL, Barrick JE, Lenski RE. 2021. Genomic evolution of antibiotic resistance is contingent on genetic background following a long-term experiment with *Escherichia coli*. *PNAS* 118(5):e2016886118
23. Cent. Dis. Control Prev. 2019. Antibiotic resistance threats in the United States, 2019. Atlanta, GA: U.S. Dept. Health Hum. Serv. **https://www.cdc.gov/antimicrobial-resistance/media/pdfs/2019-ar-threats-report-508.pdf**
24. Chambers HF, DeLeo FR. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat. Rev. Microbiol.* 7(9):629–41
25. Chen J, Quiles-Puchalt N, Chiang YN, Bacigalupe R, Fillol-Salom A, et al. 2018. Genome hypermobility by lateral transduction. *Science* 362(6411):207–12
26. Chung H, Merakou C, Schaefers MM, Flett KB, Martini S, et al. 2022. Rapid expansion and extinction of antibiotic resistance mutations during treatment of acute bacterial respiratory infections. *Nat. Commun.* 13(1):1231
27. Connor CH, Zucoloto AZ, Munnoch JT, Yu I-L, Corander J, et al. 2023. Multidrug-resistant *E. coli* encoding high genetic diversity in carbohydrate metabolism genes displace commensal *E. coli* from the intestinal tract. *PLOS Biol.* 21(10):e3002329
28. Culyba MJ, Tyne DV. 2021. Bacterial evolution during human infection: adapt and live or adapt and die. *PLOS Pathog.* 17(9):e1009872
29. Cuthbertson L, Rogers GB, Walker AW, Oliver A, Green LE, et al. 2016. Respiratory microbiota resistance and resilience to pulmonary exacerbation and subsequent antimicrobial intervention. *ISME J.* 10(5):1081–91
30. Datta MS, Yelin I, Hochwald O, Kassis I, Borenstein-Levin L, et al. 2021. Rapid methicillin resistance diversification in *Staphylococcus epidermidis* colonizing human neonates. *Nat. Commun.* 12(1):6062
31. David S, Reuter S, Harris SR, Glasner C, Feltwell T, et al. 2019. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat. Microbiol.* 4(11):1919–29
32. Davies J, Davies D. 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74(3):417–33
33. De Lastours V, Maugy E, Mathy V, Chau F, Rossi B, et al. 2017. Ecological impact of ciprofloxacin on commensal enterococci in healthy volunteers. *J. Antimicrob. Chemother.* 72(6):1574–80
34. DelaFuente J, Toribio-Celestino L, Santos-Lopez A, León-Sampedro R, Alonso-del Valle A, et al. 2022. Within-patient evolution of plasmid-mediated antimicrobial resistance. *Nat. Ecol. Evol.* 6(12):1980–91
35. Denamur E, Bonacorsi S, Giraud A, Duriez P, Hilali F, et al. 2002. High frequency of mutator strains among human uropathogenic *Escherichia coli* isolates. *J. Bacteriol.* 184(2):605–9
36. DePas WH, Starwalt-Lee R, Van Sambeek L, Ravindra Kumar S, Gradinaru V, Newman DK. 2016. Exposing the three-dimensional biogeography and metabolic states of pathogens in cystic fibrosis sputum via hydrogel embedding, clearing, and rRNA labeling. *mBio* 7(5):e00796-16
37. Diaz Caballero J, Wheatley RM, Kapel N, López-Causapé C, van der Schalk T, et al. 2023. Mixed strain pathogen populations accelerate the evolution of antibiotic resistance in patients. *Nat. Commun.* 14:4083
38. Didelot X, Walker AS, Peto TE, Crook DW, Wilson DJ. 2016. Within-host evolution of bacterial pathogens. *Nat. Rev. Microbiol.* 14(3):150–62
39. Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, et al. 2008. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. *J. Infect. Dis.* 197(11):1523–30

40. Dodson TA, Carlson EA, Wamer NC, Morse CN, Gadient JN, Prestwich EG. 2022. Characterization of distinct biofilm cell subpopulations and implications in quorum sensing and antibiotic resistance. *mBio* 13(3):e00191-22
41. 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(8):e1002837
42. Drlica K, Zhao X. 2007. Mutant selection window hypothesis updated. *Clin. Infect. Dis.* 44(5):681–88
43. Dunn S, Carrilero L, Brockhurst M, McNally A. 2021. Limited and strain-specific transcriptional and growth responses to acquisition of a multidrug resistance plasmid in genetically diverse *Escherichia coli* lineages. *mSystems* 6:e00083-21
44. Eldholm V, Norheim G, von der Lippe B, Kinander W, Dahle UR, et al. 2014. Evolution of extensively drug-resistant *Mycobacterium tuberculosis* from a susceptible ancestor in a single patient. *Genome Biol.* 15(11):490
45. Enault F, Briet A, Bouteille L, Roux S, Sullivan MB, Petit M-A. 2017. Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. *ISME J.* 11(1):237–47
46. Enne VI, Livermore DM, Stephens P, Hall LM. 2001. Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. *Lancet* 357(9265):1325–28
47. Evans DR, Griffith MP, Sundermann AJ, Shutt KA, Saul MI, et al. 2020. Systematic detection of horizontal gene transfer across genera among multidrug-resistant bacteria in a single hospital. *eLife* 9:e53886
48. Fancello L, Desnues C, Raoult D, Rolain JM. 2011. Bacteriophages and diffusion of genes encoding antimicrobial resistance in cystic fibrosis sputum microbiota. *J. Antimicrob. Chemother.* 66(11):2448–54
49. Filkins LM, O'Toole GA. 2015. Cystic fibrosis lung infections: polymicrobial, complex, and hard to treat. *PLOS Pathog.* 11(12):e1005258
50. Fisher RA, Gollan B, Helaine S. 2017. Persistent bacterial infections and persister cells. *Nat. Rev. Microbiol.* 15(8):453–64
51. Fodor AA, Klem ER, Gilpin DF, Elborn JS, Boucher RC, et al. 2012. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. *PLOS ONE* 7(9):e45001
52. Forde BM, Roberts LW, Phan M-D, Peters KM, Fleming BA, et al. 2019. Population dynamics of an *Escherichia coli* ST131 lineage during recurrent urinary tract infection. *Nat. Commun.* 10:3643
53. Fuzi M, Rodriguez Baño J, Toth A. 2020. Global evolution of pathogenic bacteria with extensive use of fluoroquinolone agents. *Front. Microbiol.* 11:504697
54. Gifford DR, Berríos-Caro E, Joerres C, Suñé M, Forsyth JH, et al. 2023. Mutators can drive the evolution of multi-resistance to antibiotics. *PLOS Genet.* 19(6):e1010791
55. Gillings MR. 2014. Integrons: past, present, and future. *Microbiol. Mol. Biol. Rev.* 78(2):257–77
56. Gillings MR, Gaze WH, Pruden A, Smalla K, Tiedje JM, Zhu Y-G. 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J.* 9(6):1269–79
57. Goig GA, Menardo F, Salaam-Dreyer Z, Dippenaar A, Streicher EM, et al. 2023. Effect of compensatory evolution in the emergence and transmission of rifampicin-resistant *Mycobacterium tuberculosis* in Cape Town, South Africa: a genomic epidemiology study. *Lancet Microbe* 4(7):e506–15
58. Gumpert H, Kubicek-Sutherland JZ, Porse A, Karami N, Munck C, et al. 2017. Transfer and persistence of a multi-drug resistance plasmid *in situ* of the infant gut microbiota in the absence of antibiotic treatment. *Front. Microbiol.* 8:1852
59. Habets MGJL, Brockhurst MA. 2012. Therapeutic antimicrobial peptides may compromise natural immunity. *Biol. Lett.* 8(3):416–18
60. Hall JPJ, Wood AJ, Harrison E, Brockhurst MA. 2016. Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. *PNAS* 113(29):8260–65
61. 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(9):1432–42
62. Herren CM, Baym M. 2022. Decreased thermal niche breadth as a trade-off of antibiotic resistance. *ISME J.* 16(7):1843–52

63. Hocquet D, Llanes C, Thouverez M, Kulasekara HD, Bertrand X, et al. 2012. Evidence for induction of integron-based antibiotic resistance by the SOS response in a clinical setting. *PLOS Pathog.* 8(6):e1002778
64. Hol FJH, Hubert B, Dekker C, Keymer JE. 2016. Density-dependent adaptive resistance allows swimming bacteria to colonize an antibiotic gradient. *ISME J.* 10(1):30–38
65. Hu Y, Yang X, Qin J, Lu N, Cheng G, et al. 2013. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nat. Commun.* 4(1):2151
66. Hubbard ATM, Jafari NV, Feasey N, Rohn JL, Roberts AP. 2019. Effect of environment on the evolutionary trajectories and growth characteristics of antibiotic-resistant *Escherichia coli* mutants. *Front. Microbiol.* 10:2001
67. Hughes D, Andersson DI. 2017. Evolutionary trajectories to antibiotic resistance. *Annu. Rev. Microbiol.* 71:579–96
68. Huo W, Busch LM, Hernandez-Bird J, Hamami E, Marshall CW, et al. 2022. Immunosuppression broadens evolutionary pathways to drug resistance and treatment failure during *Acinetobacter baumannii* pneumonia in mice. *Nat. Microbiol.* 7(6):796–809
69. Imamovic L, Sommer MOA. 2013. Use of collateral sensitivity networks to design drug cycling protocols that avoid resistance development. *Sci. Transl. Med.* 5(204):204ra132
70. Jangir PK, Ogunlana L, Szili P, Czikkely M, Shaw LP, et al. 2023. The evolution of colistin resistance increases bacterial resistance to host antimicrobial peptides and virulence. *eLife* 12:e84395
71. Jensen LK, Bjarnsholt T, Kragh KN, Aalbæk B, Henriksen NL, et al. 2019. *In vivo* gentamicin susceptibility test for prevention of bacterial biofilms in bone tissue and on implants. *Antimicrob. Agents Chemother.* 63(2):e01889-18
72. Jiao YJ, Baym M, Veres A, Kishony R. 2016. Population diversity jeopardizes the efficacy of antibiotic cycling. bioRxiv 082107. **https://doi.org/10.1101/082107**
73. Johnsborg O, Eldholm V, Håvarstein LS. 2007. Natural genetic transformation: prevalence, mechanisms and function. *Res. Microbiol.* 158(10):767–78
74. Jorth P, Staudinger BJ, Wu X, Hisert K, Hayden H, et al. 2015. Regional isolation drives bacterial diversification within cystic fibrosis lungs. *Cell Host Microbe* 18(3):307–19
75. Karami N, Martner A, Enne VI, Swerkersson S, Adlerberth I, Wold AE. 2007. Transfer of an ampicillin resistance gene between two *Escherichia coli* strains in the bowel microbiota of an infant treated with antibiotics. *J. Antimicrob. Chemother.* 60(5):1142–45
76. Karslake J, Maltas J, Brumm P, Wood KB. 2016. Population density modulates drug inhibition and gives rise to potential bistability of treatment outcomes for bacterial infections. *PLOS Comput. Biol.* 12(10):e1005098
77. Kendall EA, Fofana MO, Dowdy DW. 2015. Burden of transmitted multidrug resistance in epidemics of tuberculosis: a transmission modelling analysis. *Lancet Respir. Med.* 3(12):963–72
78. Kent AG, Vill AC, Shi Q, Satlin MJ, Brito IL. 2020. Widespread transfer of mobile antibiotic resistance genes within individual gut microbiomes revealed through bacterial Hi-C. *Nat. Commun.* 11(1):4379
79. Kim S, Lieberman TD, Kishony R. 2014. Alternating antibiotic treatments constrain evolutionary paths to multidrug resistance. *PNAS* 111(40):14494–99
80. Klümper U, Recker M, Zhang L, Yin X, Zhang T, et al. 2019. Selection for antimicrobial resistance is reduced when embedded in a natural microbial community. *ISME J.* 13(12):2927–37
81. Laborda P, Martínez JL, Hernando-Amado S. 2022. Evolution of habitat-dependent antibiotic resistance in *Pseudomonas aeruginosa*. *Microbiol. Spectr.* 10(4):e00247-22
82. Lázár V, Pal Singh G, Spohn R, Nagy I, Horváth B, et al. 2013. Bacterial evolution of antibiotic hypersensitivity. *Mol. Syst. Biol.* 9(1):700
83. Le Thomas I, Couetdic G, Clermont O, Brahimi N, Plésiat P, Bingen E. 2001. *In vivo* selection of a target/efflux double mutant of *Pseudomonas aeruginosa* by ciprofloxacin therapy. *J. Antimicrob. Chemother.* 48(4):553–55
84. Lehtinen S, Blanquart F, Croucher NJ, Turner P, Lipsitch M, Fraser C. 2017. Evolution of antibiotic resistance is linked to any genetic mechanism affecting bacterial duration of carriage. *PNAS* 114(5):1075–80

85. León-Sampedro R, DelaFuente J, Díaz-Agero C, Crellen T, Musicha P, et al. 2021. Pervasive transmission of a carbapenem resistance plasmid in the gut microbiota of hospitalized patients. *Nat. Microbiol.* 6(5):606–16
86. Levert M, Zamfir O, Clermont O, Bouvet O, Lespinats S, et al. 2010. Molecular and evolutionary bases of within-patient genotypic and phenotypic diversity in *Escherichia coli* extraintestinal infections. *PLOS Pathog.* 6(9):e1001125
87. Lieberman TD, Flett KB, Yelin I, Martin TR, McAdam AJ, et al. 2014. Genetic variation of a bacterial pathogen within individuals with cystic fibrosis provides a record of selective pressures. *Nat. Genet.* 46(1):82–87
88. Lieberman TD, Wilson D, Misra R, Xiong LL, Moodley P, et al. 2016. Genomic diversity in autopsy samples reveals within-host dissemination of HIV-associated *Mycobacterium tuberculosis*. *Nat. Med.* 22(12):1470–74
89. Lipsitch M, Moxon E. 1997. Virulence and transmissibility of pathogens: What is the relationship? *Trends Microbiol.* 5(1):31–37
90. Lipsitch M, Samore MH. 2002. Antimicrobial use and antimicrobial resistance: a population perspective. *Emerg. Infect. Dis.* 8(4):347–54
91. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, et al. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16(2):161–68
92. Long H, Miller SF, Strauss C, Zhao C, Cheng L, et al. 2016. Antibiotic treatment enhances the genome-wide mutation rate of target cells. *PNAS* 113(18):E2498–505
93. Lopatkin AJ, Meredith HR, Srimani JK, Pfeiffer C, Durrett R, You L. 2017. Persistence and reversal of plasmid-mediated antibiotic resistance. *Nat. Commun.* 8(1):1689
94. Luangtongkum T, Shen Z, Seng VW, Sahin O, Jeon B, et al. 2012. Impaired fitness and transmission of macrolide-resistant *Campylobacter jejuni* in its natural host. *Antimicrob. Agents Chemother.* 56(3):1300–8
95. Ludden C, Raven KE, Jamrozy D, Gouliouris T, Blane B, et al. 2019. One Health genomic surveillance of *Escherichia coli* demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. *mBio* 10:e02693-18
96. Lukačišinová M, Fernando B, Bollenbach T. 2020. Highly parallel lab evolution reveals that epistasis can curb the evolution of antibiotic resistance. *Nat. Commun.* 11(1):3105
97. Markussen T, Marvig RL, Gómez-Lozano M, Aanæs K, Burleigh AE, et al. 2014. Environmental heterogeneity drives within-host diversification and evolution of *Pseudomonas aeruginosa*. *mBio* 5(5):e01592-14
98. Martinez JL, Baquero F. 2000. Mutation frequencies and antibiotic resistance. *Antimicrob. Agents Chemother.* 44(7):1771–77
99. Mathers AJ, Peirano G, Pitout JDD. 2015. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clin. Microbiol. Rev.* 28(3):565–91
100. McCarthy AJ, Loeffler A, Witney AA, Gould KA, Lloyd DH, Lindsay JA. 2014. Extensive horizontal gene transfer during *Staphylococcus aureus* co-colonization in vivo. *Genome Biol. Evol.* 6(10):2697–708
101. Melnyk AH, Wong A, Kassen R. 2015. The fitness costs of antibiotic resistance mutations. *Evol. Appl.* 8(3):273–83
102. Modi SR, Lee HH, Spina CS, Collins JJ. 2013. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. *Nature* 499(7457):219–22
103. Moradigaravand D, Gouliouris T, Blane B, Naydenova P, Ludden C, et al. 2017. Within-host evolution of *Enterococcus faecium* during longitudinal carriage and transition to bloodstream infection in immunocompromised patients. *Genome Med.* 9(1):119
104. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, et al. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399(10325):629–55
105. Mwangi MM, Wu SW, Zhou Y, Sieradzki K, de Lencastre H, et al. 2007. Tracking the *in vivo* evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. *PNAS* 104(22):9451–56
106. Nazarian P, Tran F, Boedicker JQ. 2018. Modeling multispecies gene flow dynamics reveals the unique roles of different horizontal gene transfer mechanisms. *Front. Microbiol.* 9:416876

107. Neil K, Allard N, Grenier F, Burrus V, Rodrigue S. 2020. Highly efficient gene transfer in the mouse gut microbiota is enabled by the $Incl_2$ conjugative plasmid TP114. *Commun. Biol.* 3(1):523
108. Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. 2014. *Escherichia coli* ST131, an intriguing clonal group. *Clin. Microbiol. Rev.* 27(3):543–74
109. 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(3):504–14
110. O'Brien S, Baumgartner M, Hall AR. 2021. Species interactions drive the spread of ampicillin resistance in human-associated gut microbiota. *Evol. Med. Public Health* 9(1):256–66
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(5469):1251–53
112. Oliver A, Mulet X, López-Causapé C, Juan C. 2015. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. *Drug Resist. Updates* 21:41–59
113. Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile genetic elements associated with antimicrobial resistance. *Clin. Microbiol. Rev.* 31(4):e00088-17
114. Pathak A, Angst DC, León-Sampedro R, Hall AR. 2023. Antibiotic-degrading resistance changes bacterial community structure via species-specific responses. *ISME J.* 17(9):1495–503
115. Peter S, Bosio M, Gross C, Bezdan D, Gutierrez J, et al. 2020. Tracking of antibiotic resistance transfer and rapid plasmid evolution in a hospital setting by nanopore sequencing. *mSphere* 5:e00525-20
116. Pfeifer E, Bonnin RA, Rocha EPC. 2022. Phage-plasmids spread antibiotic resistance genes through infection and lysogenic conversion. *mBio* 13(5):e01851-22
117. Pitout JDD, Peirano G, Chen L, DeVinney R, Matsumura Y. 2022. *Escherichia coli* ST1193: following in the footsteps of *E. coli* ST131. *Antimicrob. Agents Chemother.* 66(7):e00511-22
118. Planet PJ. 2017. Life after USA300: the rise and fall of a superbug. *J. Infect. Dis.* 215(Suppl. 1):S71–77
119. Prasad NK, Seiple IB, Cirz RT, Rosenberg OS. 2022. Leaks in the pipeline: a failure analysis of Gram-negative antibiotic development from 2010 to 2020. *Antimicrob. Agents Chemother.* 66(5):e00054-22
120. Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys J-P. 2006. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science* 313(5783):89–92
121. Pujol M, Peña C, Pallares R, Ariza J, Ayats J, et al. 1996. Nosocomial *Staphylococcus aureus* bacteremia among nasal carriers of methicillin-resistant and methicillin-susceptible strains. *Am. J. Med.* 100(5):509–16
122. Quirós P, Colomer-Lluch M, Martínez-Castillo A, Miró E, Argente M, et al. 2014. Antibiotic resistance genes in the bacteriophage DNA fraction of human fecal samples. *Antimicrob. Agents Chemother.* 58(1):606–9
123. Rau MH, Marvig RL, Ehrlich GD, Molin S, Jelsbak L. 2012. Deletion and acquisition of genomic content during early stage adaptation of *Pseudomonas aeruginosa* to a human host environment. *Environ. Microbiol.* 14(8):2200–11
124. Robinson DA, Enright MC. 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 47(12):3926–34
125. Rodríguez-Beltrán J, Sørum V, Toll-Riera M, de la Vega C, Peña-Miller R, San Millán Á. 2020. Genetic dominance governs the evolution and spread of mobile genetic elements in bacteria. *PNAS* 117(27):15755–62
126. Sakoulas G, Okumura CY, Thienphrapa W, Olson J, Nonejuie P, et al. 2014. Nafcillin enhances innate immune-mediated killing of methicillin-resistant *Staphylococcus aureus*. *J. Mol. Med.* 92(2):139–49
127. San Millan A, Escudero JA, Gifford DR, Mazel D, MacLean RC. 2016. Multicopy plasmids potentiate the evolution of antibiotic resistance in bacteria. *Nat. Ecol. Evol.* 1(1):0010
128. San Millan A, MacLean RC. 2017. Fitness costs of plasmids: a limit to plasmid transmission. *Microbiol. Spectr.* 5(5):MTBP0016-2017
129. Santos-Lopez A, Marshall CW, Scribner MR, Snyder DJ, Cooper VS. 2019. Evolutionary pathways to antibiotic resistance are dependent upon environmental structure and bacterial lifestyle. *eLife* 8:e47612
130. Sarkar S, Hutton ML, Vagenas D, Ruter R, Schüller S, et al. 2018. Intestinal colonization traits of pandemic multidrug-resistant *Escherichia coli* ST131. *J. Infect. Dis.* 218(6):979–90

131. Schaufler K, Semmler T, Pickard DJ, de Toro M, de la Cruz F, et al. 2016. Carriage of extended-spectrum beta-lactamase-plasmids does not reduce fitness but enhances virulence in some strains of pandemic *E. coli* lineages. *Front. Microbiol.* 7:336
132. Selgrad M, Tammer I, Langner C, Bornschein J, Meißle J, et al. 2014. Different antibiotic susceptibility between antrum and corpus of the stomach, a possible reason for treatment failure of *Helicobacter pylori* infection. *World J. Gastroenterol.* 20(43):16245–51
133. Sentausa E, Basso P, Berry A, Adrait A, Bellement G, et al. 2019. Insertion sequences drive the emergence of a highly adapted human pathogen. *Microb. Genom.* 6(9):mgen000265
134. Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL. 2003. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *CHEST* 123(5):1495–502
135. Sommer MOA, Dantas G, Church GM. 2009. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science* 325(5944):1128–31
136. Souque C, Escudero JA, MacLean RC. 2021. Integron activity accelerates the evolution of antibiotic resistance. *eLife* 10:e62474
137. Stecher B, Denzler R, Maier L, Bernet F, Sanders MJ, et al. 2012. Gut inflammation can boost horizontal gene transfer between pathogenic and commensal *Enterobacteriaceae*. *PNAS* 109(4):1269–74
138. Stevanovic M, Boukéké-Lesplulier T, Hupe L, Hasty J, Bittihn P, Schultz D. 2022. Nutrient gradients mediate complex colony-level antibiotic responses in structured microbial populations. *Front. Microbiol.* 13:740259
139. Stevenson C, Hall JP, Harrison E, Wood AJ, Brockhurst MA. 2017. Gene mobility promotes the spread of resistance in bacterial populations. *ISME J.* 11(8):1930–32
140. Stoesser N, Sheppard AE, Pankhurst L, De Maio N, Moore CE, et al. 2016. Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. *mBio* 7(2):e02162
141. Stracy M, Snitser O, Yelin I, Amer Y, Parizade M, et al. 2022. Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. *Science* 375(6583):889–94
142. Sukhum KV, Newcomer EP, Cass C, Wallace MA, Johnson C, et al. 2022. Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. *Commun. Med.* 2(1):62
143. Sun G, Luo T, Yang C, Dong X, Li J, et al. 2012. Dynamic population changes in *Mycobacterium tuberculosis* during acquisition and fixation of drug resistance in patients. *J. Infect. Dis.* 206(11):1724–33
144. Sun L, Alexander HK, Bogos B, Kiviet DJ, Ackermann M, Bonhoeffer S. 2018. Effective polyploidy causes phenotypic delay and influences bacterial evolvability. *PLOS Biol.* 16(2):e2004644
145. Sundqvist M, Geli P, Andersson DI, Sjolund-Karlsson M, Runehagen A, et al. 2010. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. *J. Antimicrob. Chemother.* 65(2):350–60
146. Szybalski W, Bryson V. 1952. Genetic studies on microbial cross resistance to toxic agents. I. Cross resistance of *Escherichia coli* to fifteen antibiotics. *J. Bacteriol.* 64(4):489–99
147. Tedijanto C, Olesen SW, Grad YH, Lipsitch M. 2018. Estimating the proportion of bystander selection for antibiotic resistance among potentially pathogenic bacterial flora. *PNAS* 115(51):E11988–95
148. Thänert R, Reske KA, Hink T, Wallace MA, Wang B, et al. 2019. Comparative genomics of antibiotic-resistant uropathogens implicates three routes for recurrence of urinary tract infections. *mBio* 10(4):e01977-19
149. Theuretzbacher U, Bush K, Harbarth S, Paul M, Rex JH, et al. 2020. Critical analysis of antibacterial agents in clinical development. *Nat. Rev. Microbiol.* 18(5):286–98
150. Thurlow LR, Joshi GS, Richardson AR. 2012. Virulence strategies of the dominant USA300 lineage of community associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *FEMS Immunol. Med. Microbiol.* 65(1):5–22
151. Tonkin-Hill G, Ling C, Chaguza C, Salter SJ, Hinfonthong P, et al. 2022. Pneumococcal within-host diversity during colonization, transmission and treatment. *Nat. Microbiol.* 7(11):1791–804
152. Traglia GM, Place K, Dotto C, Fernandez JS, Montaña S, et al. 2019. Interspecies DNA acquisition by a naturally competent *Acinetobacter baumannii* strain. *Int. J. Antimicrob. Agents* 53(4):483–90

153. van Duijn PJ, Verbrugghe W, Jorens PG, Spöhr F, Schedler D, et al. 2018. The effects of antibiotic cycling and mixing on antibiotic resistance in intensive care units: a cluster-randomised crossover trial. *Lancet Infect. Dis.* 18(4):401–9
154. Vega NM, Gore J. 2014. Collective antibiotic resistance: mechanisms and implications. *Curr. Opin. Microbiol.* 21:28–34
155. Vogwill T, Kojadinovic M, MacLean RC. 2016. Epistasis between antibiotic resistance mutations and genetic background shape the fitness effect of resistance across species of *Pseudomonas*. *Proc. R. Soc. B Biol. Sci.* 283(1830):20160151
156. Vogwill T, MacLean RC. 2015. The genetic basis of the fitness costs of antimicrobial resistance: a meta-analysis approach. *Evol. Appl.* 8(3):284–95
157. Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, et al. 2018. The global distribution and spread of the mobilized colistin resistance gene *mcr-1*. *Nat. Commun.* 9(1):1179
158. Wheatley R, Diaz Caballero J, Kapel N, de Winter FHR, Jangir P, et al. 2021. Rapid evolution and host immunity drive the rise and fall of carbapenem resistance during an acute *Pseudomonas aeruginosa* infection. *Nat. Commun.* 12(1):2460
159. Wheatley RM, Caballero JD, van der Schalk TE, De Winter FHR, Shaw LP, et al. 2022. Gut to lung translocation and antibiotic mediated selection shape the dynamics of *Pseudomonas aeruginosa* in an ICU patient. *Nat. Commun.* 13(1):6523
160. Willems RJL, Hanage WP, Bessen DE, Feil EJ. 2011. Population biology of Gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. *FEMS Microbiol. Rev.* 35(5):872
161. Williams D, Fothergill JL, Evans B, Caples J, Haldenby S, et al. 2018. Transmission and lineage displacement drive rapid population genomic flux in cystic fibrosis airway infections of a *Pseudomonas aeruginosa* epidemic strain. *Microb. Genom.* 4(3):e000167
162. Willmann M, Vehreschild MJGT, Biehl LM, Vogel W, Dörfel D, et al. 2019. Distinct impact of antibiotics on the gut microbiome and resistome: a longitudinal multicenter cohort study. *BMC Biol.* 17(1):76
163. 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(5):736–55
164. Woods LC, Gorrell RJ, Taylor F, Connallon T, Kwok T, McDonald MJ. 2020. Horizontal gene transfer potentiates adaptation by reducing selective constraints on the spread of genetic variation. *PNAS* 117(43):26868–75
165. Worby CJ, Sridhar S, Turbett SE, Becker MV, Kogut L, et al. 2023. Gut microbiome perturbation, antibiotic resistance, and *Escherichia coli* strain dynamics associated with international travel: a metagenomic analysis. *Lancet Microbe* 4(10):e790–99
166. Wyres KL, Lam MMC, Holt KE. 2020. Population genomics of *Klebsiella pneumoniae*. *Nat. Rev. Microbiol.* 18(6):344–59
167. Yang QE, Walsh TR. 2017. Toxin-antitoxin systems and their role in disseminating and maintaining antimicrobial resistance. *FEMS Microbiol. Rev.* 41(3):343–53
168. Yassour M, Vatanen T, Siljander H, Hämäläinen A-M, Härkönen T, et al. 2016. Natural history of the infant gut microbiome and impact of antibiotic treatments on strain-level diversity and stability. *Sci. Transl. Med.* 8(343):343ra81
169. Young BC, Wu C-H, Gordon NC, Cole K, Price JR, et al. 2017. Severe infections emerge from commensal bacteria by adaptive evolution. *eLife* 6:e30637
170. Yurtsev EA, Chao HX, Datta MS, Artemova T, Gore J. 2013. Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. *Mol. Syst. Biol.* 9:683
171. Zhai Y, Pribis JP, Dooling SW, Garcia-Villada L, Minnick PJ, et al. 2023. Drugging evolution of antibiotic resistance at a regulatory network hub. *Sci. Adv.* 9(25):eadg0188
172. Zhou J, Dong Y, Zhao X, Lee S, Amin A, et al. 2000. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. *J. Infect. Dis.* 182(2):517–25