Ecological Kinetics and Evolutionary Dynamics of Antibiotic Resistance in Complex Environments

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Abstract

Antibiotic-resistant bacteria are common in the natural environment, including the microbiota of animal and human hosts. The local physical, chemical, and biological conditions of environmental patches and matrices vary in both qualitative and quantitative aspects. Often, these conditions diffuse in gradients, creating intersections that can either facilitate or inhibit the spread and evolution of antibiotic resistance (AbR). Ecological kinetics (EcoK) describes how these conditions and their combinations influence the abundance and transmission of antibiotic-resistant populations, as well as the mobile genetic elements that carry resistance genes. Evolutionary dynamics (EvoD) concern the effects of environmental conditions on genetic variation, the expression of resistance traits, and the evolvability of host bacteria, ultimately contributing to the emergence and selection of AbR. This study examines the influence of various environmental conditions, including temperature, oxygen availability, fluidity, humidity, osmolarity, acidity, particulate material, nutrients, organic matter, microbial density, and the presence of metals, pharmaceutical substances, biocides, pesticides, antibiotics, and antimicrobials produced by phytoplankton, plants, and animals, on EcoK and EvoD. This field remains scarcely understood, and further research is essential to identify high-risk areas where surveillance for the emergence and spread of AbR should be implemented.

Contents

1. INTRODUCTION

The evolution and transmission of bacterial phenotypes, including antibiotic resistance (AbR) occur within a two-dimensional framework of space and time, possibly a space-time continuum. The varying conditions of Earth's habitable space undergo selection for bacterial populations, leading to changes in local abundance over time, resulting in genetic variation. Spatial selection relies on the potential for bacterial migration as well as the heterogeneity of spaces and environmental matrices. Selective spaces differ not only in size but also in their physical, chemical, and biological compositions, driving genetic diversification. These conditions exhibit varying intensities across space and time, and the gradients of each component intersect, creating a multi-component selective network that influences a vast multiplicity of resistance phenotypes. Specific tessellated spaces resulting from intersections hold greater significance, facilitating and selecting various phenotypic pathways and trajectories that differ in robustness and evolvability (16, 79). We are interested in analyzing the effects of the physical, chemical, and biological components of a landscape that contribute to shaping the ecological kinetics and evolutionary dynamics of AbR, as defined below. Our primary conclusion is that AbR emerges, is expressed, and spreads, driven by a vast interconnected network of ecological traits, particularly when acting in combination with antibiotic exposure.

In this work, the term **ecological kinetics** (EcoK) refers to the various spaces colonized by microbial organisms that undergo change and how this temporal heterogeneity influences the transmission of antibiotic-resistance genes (ARGs) within and between cells, populations, and communities or the transfer of resistant bacterial cells between different ecological spaces. Following source-sink dynamics, variations in habitat quality may affect resistance to population growth or decline. Local EcoK relies on local sources that shape fitness and the abundance of

antibiotic-resistant bacteria (52, 123). Spaces near hotspots of resistant organisms, such as farms, human and farm sewage, including hospital, municipal, household, and aquaculture effluents, or those resulting from agricultural technologies like composting, act as sources of resistant genes and populations. However, other spaces do not promote the transmission of resistance, serving as resistance sinks. The term **evolutionary dynamics** (EvoD) refers to how ecological changes in defined compartments influence genetic variation, the expression of resistance traits, and the evolvability of the hosted bacteria, leading to the emergence and selection of antibiotic resistance (AbR) and its progress over time toward more efficient mechanisms—such as increasing resistance levels, offering a broader spectrum of antibiotic inactivation, and incurring lower fitness costs (112). Similar to EcoK, EvoD is triggered by a wide range of local conditions. EcoK and EvoD are necessarily intertwined; thus, the genetic evolution of microorganisms can alter the composition of these ecological spaces, triggering genetic and phenotypic diversification that results in contingent yet continuously changing interactions between EcoK and EvoD.

ECOLOGICAL KINETICS

Ecological kinetics is the study of environmental changes that alter the conditions of colonized spaces, leading to fluctuations in the abundance, diversity, and transmission of antibiotic resistance genes (ARGs) and their associated populations. In this work, changes in the host's spaces are also considered. Microbial transmission also occurs inside the host, which is composed of a series of spatially differentiated but connected environments (143). The effects on ARGs and bacterial populations result from both macro- and microenvironmental variables that shape the sources and transport of resistance (48, 78). In each of the sections below, we begin with the impact of changes in environmental conditions on the density and transmission of bacterial populations, followed by an examination of how these changes modify the transmission of AbR determinants.

2.1. **Temperature**

Even after brief periods of exposure to elevated mean temperatures, whether from daily temperature fluctuations or short-term heatwaves, the intestinal microbiota of mammals, reptiles, amphibians, and birds shows a reduction in Firmicutes and Actinobacteria, and an increase in Proteobacteria (136). Hot summer weather is linked to a higher prevalence of Gram-negative bacterial infections (119). Temperature can also rise within an individual host, especially due to inflammation and immune activation following infection; in addition, different animals have varying body temperatures. Bacteria possess mechanisms, such as the diguanylate signaling network, that enable them to sense temperature (3). Generally, as temperatures rise, enzymes demonstrate greater efficiency, boosting the growth rate. Overall, warmer conditions may optimize metabolic and biosynthetic adaptations, leading to a hypermetabolic state in cells and accelerating the mutation rate (82). Beyond animals, such heat-generating activity occurs during agricultural microbial composting, where animal and human waste are combined with green manure, plant matter, refuse, and soil. Bacteria are also subjected to heat in air conditioning appliances, such as bladeless electric fans (4), or heated water, which affects microbial biofilms found in colonized sinks and plumbing systems.

Microbiota alpha-diversity decreases in cold-stressed animals, accompanied by an increase in members of the Gram-positive *Lachnospiraceae* family of Firmicutes, leading to the overproduction of short-chain fatty acids with antimicrobial activity, which may orchestrate internal thermogenesis (35, 63). Among Gram-negatives, the abundance of the families *Bacteroidaceae* and *Enterobacteriaceae* is reduced under these cold conditions. However, rare species of the genus *Escherichia*, such as *E. marmotae*, seem relevant in the microbiota associated with hibernation in animals like bears and marmots, as well as in winter torpor in birds. An unusually high proportion of

E. marmotae strains produce the antimicrobial lasso peptide microcin J25 (MccJ25), which simplifies the microbiota, increases body weight (nutritional reserves) as an initial step in hibernation, reducing the bioenergetic demand of the animal, thereby limiting host activity by functioning as a neurotransmitter-like peptide (11), which decreases the transmission of AbR.

Within the limits imposed by the trade-off between increased enzymatic activity and fitness-reducing protein folding alterations, most microbial cells, including mesophilic ones, have a maximal growth rate peaking at 42 °C, with secondary peaks at 67 °C for thermophiles (37, 96). However, the range of temperatures sustaining a reasonably high growth rate is wide for certain organisms; for instance, cold (4-6ºC) selectively enriches *Listeria monocytogenes*, which can also grow at higher temperatures. In some cases, the same organism can adapt to both cold and warm temperatures by protecting proteins from misfolding through the activation of a chaperone (53).

The biokinetic effect of sub-inhibitory temperatures on various bacterial organisms influences the overall transmission of AbR. First, in clinically significant organisms such as Enterobacterales, the spread of these organisms and their resistance determinants is likely to increase with the temperature-driven absolute abundance of cells harboring mobile resistance elements. Second, higher ATP levels resulting from elevated temperatures provide more energy for plasmid transfer. The interbacterial transfer of different plasmids may have varying optimal temperature ranges, typically between 25 °C and 30 °C. The optimal growth temperature of the mating pair also serves as the optimal condition for plasmid transfer (58, 147). Exceptionally, such as with IncH plasmids, temperatures of 37 °C can inhibit conjugation (121). A temperature of 37ºC may elevate reactive oxygen species, triggering the SOS response and pilus channels that facilitate conjugation. Bacterial acquisition of resistance through DNA transformation also increases with temperature (57). Third, some plasmids, particularly smaller ones, relax their copy number control when

159 temperatures rise, potentially leading to enhanced AbR and eventual co-transfer with helper
160 conjugative mobile elements (128). Whether through increased bacterial abundance or horizontal
161 gene transfer (HGT), AbR escalates with local temperatures (90). These observations about
162 temperature and the transmission of AbR should be considered in light of global warming (145).

163 2.2. **Fluidity and Relative Humidity**

164 Fluids, flowing substances across denser material, facilitate the transmission of antibiotic-resistant
165 organisms and chemicals, favoring bacterial reproduction and selection. Additionally,
166 environmental wetting is essential for swimming and twitching bacterial self-motility. Fluids as
167 water or high-water products, are therefore critical in the spread of resistance (15). On the contrary,
168 high fluidity facilitates dilution and reduces the local density of resistant organisms, as observed
169 when comparing the upper, middle, and lower courses of contaminated rivers (118). A hotspot for
170 antibiotic emergence and spread is sewage and sludge (15); it cannot be ruled out that sewage
171 treatment plants may contribute to this process (9, 110). The blending of fluids and solid particles,
172 like the gut-to-mud process (66), creates water vapor (humidity) in the local air, which also
173 promotes bacterial growth on surfaces. The increased frequency of extreme precipitation events,
174 which cause catastrophic floods, disrupts limited sanitation facilities and contributes to the growth
175 and spread of AbR (51).

176 In contrast, dry conditions limit the growth and dispersal of resistant microorganisms and reduce
177 local selection by antibiotics (21). In urban areas, including hospital built-environments, fluids
178 contribute to the local biofilm formation, serving as refuges for antibiotic-resistant organisms over
179 decades, particularly water running in water pipes and drained in basin sinks (8). Biofilms maintain
180 fluidity and humidity, ensuring a spatial association of bacterial communities within a common
181 hydrated polymeric substance, which may facilitate the transfer of antibiotic-resistance genes (80).

The combination of conditions, such as mixing temperature and relative humidity, significantly enhances bacterial growth and proliferation of antibiotic-resistant bacteria. Ventilation decreases the effects of temperature (106). Storage cabinets, including those containing food, are typically enclosed, with minimal ventilation and very low interior air movement, often resulting in high humidity levels that favor bacterial multiplication, promiscuity, and HGT of resistance. The food industry, particularly in medium- to low-income countries, should be aware of the effects of storage conditions, including the use of containers designed for shipping activities. In this respect, large and closed reservoirs, such as those containing ship ballast water, have also been identified as potential risks for the evolution and spread of AbR (89).

2.3. **Acidity or Basicity**

The significance of pH as a regulator of microbial metabolism is a well-established fact in determining the abundance and location of specific bacterial species. pH influences both the integrity of cell structures and metabolism, including microbial respiration. In natural environments, it is estimated that a decrease or increase in environmental pH by one unit can reduce the metabolic activity of microbial communities by up to 50% (69). Studies of soil pH gradients demonstrate that the relative abundance and diversity of bacteria positively correlate with pH, nearly doubling between pH 4 and 8 (115). The populations of acidophiles, neutrophiles, and alkaliphiles, which depend on optimal pH for growth, are ecologically separated, but only at extreme pH levels (69).

Klebsiella pneumoniae can thrive between pH 6 and 8, *P. aeruginosa* from 4 to 9, and *Enterococcus* from 4 to 10. It is noteworthy that bacteria growing under highly acidic conditions are not necessarily acidophiles, as illustrated by *Helicobacter pylori*, which possesses mechanisms to survive stomach acidity. A similar case is *Escherichia coli*, which grows optimally at pH 7.2-

7.8 but can thrive in a pH range from 5 or lower in the stomach to 9 in the pancreatic duct (26). Fertilization regimes influence soil pH and the trajectory of ARGs (141). Plasmid transfer may be modulated by pH, but the information is scarce and likely variable depending on the type of plasmid (70, 72). For example, it has been demonstrated that a plasmid containing a CTX-M beta-lactamase is inhibited from transferring at pH 9 (5).

2.4. Osmolarity, salinity

Osmotic stress decreases enzymatic activity, leading to reduced bacterial growth and expression of resistance genes (7). This likely occurs due to decreased water availability, resulting in intracellular molecular crowding, impaired protein folding, and altered protein-nucleic acid interactions (139). These biophysical effects influence subcellular structure, with spatial (topological) changes driven by variations in the quantity or local density of molecules within cell compartments. Such structural alterations may lead to structural epistatic phenotypes associated with AbR (17). However, most bacterial species possess osmoregulatory protective mechanisms, often utilizing proline and glycine betaine to alleviate osmotic pressure (31). Conversely, hypo-osmotic stress can occur in *E. coli* when it is expelled from the host intestine into a diluted external environment (32). This stress can be mitigated by specific mechanosensitive channels that release cytoplasmic solutes, preventing the cell envelope from bursting (45).

The EcoK of many pathogenic and antibiotic-resistant microorganisms is certainly determined by environmental osmolarity. For instance, there is a clear gradient of salt concentration between feces, where halophilic species can be established, brackish water, seawater, and freshwater. The survival rate in these conditions varies significantly; for instance, the percent survival of E. coli after 48 h was estimated to range from 75% in brackish water to 8% in full-strength seawater; however, these rates can differ in different ecological sites (120). The interaction between salinity

and temperature, modulated by nutrient availability, shapes the distribution of the resistome across geographic regions in the sea (120).

Plasmid conjugation frequency in non-growing cultures in 9.1mM NaCl is similar to that in LB broth growing cells, but much higher than in non-growing cells in 1mM or 90.1mM NaCl. The reason may be that salinity induces conformational changes in cell surface macromolecules, thereby altering cell-cell interactions (58).

2.5. Oxygen availability

Microbial oxygen availability is primarily determined by direct contact of this element with surfaces exposed to the atmosphere, the rate of diffusion in soil, depending on soil porosity, and dissolved oxygen in freshwater, including aquifers, groundwater, lakes, rivers, drinking water, wastewater, and seawater. This availability varies with turbulence and distance to the atmosphere. A reduction in oxygen availability is influenced by local oxygen consumption, mainly through chemical oxidation and respiration, often aided by local aerobic or facultative microbes found in the gut, soils, and water bodies, especially those enriched in organic matter like sewage. Areas of anoxic waters mainly result from local eutrophication due to the discharge of nitrates and phosphates from farmlands, aquaculture ponds, or urban and industrial zones, which can lead to methane enrichment. The availability of local oxygen affects the bacterial composition of communities, which vary in the proportions of obligate aerobes, facultative anaerobes, strict anaerobes, aerotolerant anaerobes, and microaerophilic species. In human feces, anaerobic organisms outnumber aerobic and facultative anaerobic microbes by about 1,000 times; in surface soil, aerobic and facultative microbes are predominant, while anaerobic microbes are more common in deep soil, particularly in compacted or hydric soils, within soil particles. The effectiveness of

wastewater treatment plants may benefit from either aerobic-facultative or anaerobic bacteria, depending on whether the treatment is aerobic, which is more frequent in high-income countries, or anaerobic. Flowing rivers favor aerobic-facultative bacteria, although frequent biofilm formation may help maintain the persistence of anaerobic organisms. In estuaries, aerobic anoxygenic phototrophic bacteria, primarily belonging to the Alpha, Beta, and Gammaproteobacteria groups, tend to dominate. In the ocean, the balance of aerobic and anaerobic conditions depends on the distance from the surface and the depth of the water column. In oxygenated subseafloor sediment, facultative Gammaproteobacteria and Firmicutes are predominant. In contrast, anaerobic organisms from the groups *Atribacteria, Chloroflexi, Planctomycetes* are prevalent in anoxic sediments (60). However, clinically relevant resistance does not significantly increase with exposure to antibiotics in marine sediments (141). Relatively low bacterial densities, along with phylogenetic and ecological barriers, complicate predictions about the transmission of these populations. Plasmids in bacterial populations in water bodies might also belong to different plasmid taxonomic units (PTUs), preventing mutual genetic interactions. Additionally, in wastewater ecosystems, gene transfer frequencies tend to be lower in the presence of oxygen (92). Moreover, plasmids found in marine sediment microbial communities appear to differ from those harboring ARGs (124). An open field of research explores the connectivity of bacterial gasoreceptors that detect oxygen, exemplified by the phosphodiesterase DosP in *E. coli.* Additionally, the FeS cluster-containing transcription factors FNR and NsrR, which also detect nitric oxide (6), play a role in this area. The resulting regulation of bacterial metabolism impacts both growth and genetic transfer.

2.6. **Biofilms and particulate material**

Biofilms, which are social consortia of cells adhered to surfaces and embedded in an extracellular matrix, represent the most common forms of bacterial coexistence in nature, as often contain

various species. In ecological kinetics, biofilm formation serves multiple, sometimes contradictory roles. On one hand, it decreases the transmission rate among ecological compartments since bacteria are fixed within the matrix and replicate at a very low rate. However, fragments of these biofilms, with a high inoculum, can detach and potentially colonize other surfaces more efficiently than planktonic bacteria, thereby ensuring the transmission and persistence of cooperative consortia. Biofilms, such as those found in *P. aeruginosa*, reduce ecological competition among different species in adjacent patches by releasing antibacterial substances, particularly under stress conditions (101). Among the many physical substrates for biofilms, mineral particles, such as clay, carbonates, and silicates, as well as anthropogenic materials, including wastewater particles, and microplastics should be considered. Other physical surfaces are provided by phytoplankton, zooplankton, humus, and various forms of biodetritus. Water flow, tides, tsunamis, currents, and strong winds, along with agriculture, wastewater management, mining, excavation, and construction, facilitate the interaction and merging of microbiotic particles in soil and water, leading to enhanced coalescence of heterogeneous biofilm communities capable of exchanging genetic material, including plasmids harboring antimicrobial resistance genes (12). These complex biofilms also arise from the merging of a patient's microbiota with hospital-associated microbiota, such as that found in water sinks, and have been suggested as reservoirs of AbR in intensive care units (8). In hospital sinks, whether using cold or warm water, the metal of the pipelines, or introduced disinfectants and drug residues, can induce bacterial stress, promoting plasmid persistence and conjugation in local biofilms (54, 130). Although biofilms are often considered hotspots for HGT, several studies have indicated that plasmid transfer is primarily restricted to the outer layers (58). In turn, plasmids contribute to biofilm production and expansion (125). In their turn, plasmids contribute to biofilm production and expansion (111).

296 2.7. **Organic matter, nutrients**

297 Ecological adaptation implies that genotypes should be expressed in adaptive phenotypes. This
298 process is influenced by variation in concentration of organic matter and nutrients (146).
299 Nutritional deficiency reduces the number of ribosomes and, therefore, the bacterial growth rate.
300 The reduction in ribosomal number contributes to a vicious cycle in the synthesis of critical
301 nutrients. Such variations lead to decoupled relationships between carbohydrate-active enzymes
302 and peptidases from genetic potentials involved in protein expressions (146). The ecological
303 consequences of nutrient availability have been proposed to result from biological stoichiometry,
304 that is, the influence of the balance of multiple chemical elements, as C:N:P- stoichiometry, in
305 ecological interactions (47). The mechanism primarily involves an increased allocation to P-rich
306 rRNA, leading to rapid protein synthesis by ribosomes. Under nutrient-limited conditions, the
307 production of ribosomes tends to decrease in order to conserve energy. In dense, structured
308 populations, such as biofilms, collective nutrient consumption may limit antibiotic efficacy (55).
309 However, that might be mitigated by the local exchange of metabolites (142).

310 **Metals**

311 Metal resistance and AbR frequently result from the same type of mechanisms of cellular
312 protection, as: 1) reduced membrane permeability (metals: As, Cu, Zn, Mn, Co, Ag; antibiotics:
313 beta-lactams, fluoroquinolones, tetracycline, chloramphenicol); 2) chemical alteration of the
314 antimicrobial (metals: As, Hg; antibiotics: beta-lactams, chloramphenicol, aminoglycosides); 3)
315 efflux pumps (metals: Cu, Co, Zn, Cd, Ni; antibiotics: tetracyclines, chloramphenicol, beta-
316 lactams; 4) alteration of cellular targets (metals: Hg, Zn; antibiotics: ciprofloxacin, b-lactams,
317 macrolides, trimethoprim, rifampicin; and (5) antimicrobial sequestration (metals: Zn, Cd;

antibiotics: coumermycin A) (10). Therefore, heavy metal contamination can contribute to the dissemination of AbR.

The effect of metals on resistance plasmid transfer is heterogeneous, depending on the level of exposure, type of plasmid, and bacterial organism. $HgCl_2$ at concentrations above 1.0 mg L-1 negatively affected transfer, whereas 2,4-D at concentrations up to 0.3 mM had no effect; the negative effects of mercury were attributed to stress on the recipient (71). This effect may vary across different taxa, such as increased or decreased conjugation permissiveness by more than 1000-fold (74). In *Escherichia coli,* zinc oxide and copper sulfate increase the conjugation frequency of IncL plasmids (109).

2.9 Radiation and Lightning

Different bacterial species and genera are heterogeneous in resistance to radiation. A correlation has been found between AbR or heavy metal resistance in bacteria dominating areas contaminated with both artificial and naturally occurring radionuclides, as *Acinetobacter*, *Burkholderia*, *Streptomyces*, *Bacillus*, and *Staphylococcus.* Bacteria with yellow, orange, red, or pink colonies, mainly due to carotenoids, show higher resistance to radiation (22). Radiation at the UV region (265 nm) was most efficient at killing the *E. coli* cells. However, even in the visible light spectrum, a reduction in *E. coli* viability also occurs. Viability decreases exponentially with the increase in radiation dosage at a given wavelength (129).

In aquatic environments, the conjugative transfer frequency of AbR was unaffected by visible light irradiation but was slightly increased (2–10-fold) by simulated sunlight irradiation and significantly accelerated (up to 100-fold) by UV irradiation. These results are likely due to oxidative stress (33).

Pharmaceuticals, biocides, pesticides, antibiotics

Many non-antibiotic pharmaceuticals are present in the environment. The most frequently detected molecules in river water include carbamazepine, metformin, and caffeine (135). Carbazepine influences host-bacterial interactions; metformin belongs to the molecular class of biguanides, which eventually selects for AbR; caffeine has antimicrobial activity. Biocides certainly decrease local bacterial abundance and, at subinhibitory concentrations, select for AbR mutations (134). Antimicrobial pesticides, such as endosulfan, carbofuran, and malathion, favor bacterial pesticide degraders and are likely to have a higher potential for antimicrobial resistance by using similar mechanisms of detoxification (108). The key role of antibiotics in selecting for resistant microorganisms, whether in individuals or the environment, even at very low antibiotic concentrations, has been extensively reviewed and will not be addressed here (15, 137). Mycotoxins, an extensive group of molecules produced by fungi, share a close structural relationship with antibiotics; in fact, many natural antibiotics and mycotoxins are conceptually distinguished by the therapeutic utility of the former. As a result, mycotoxins often exhibit antibacterial activity and may locally select for antibiotic-resistant organisms (77).

It is generally accepted that the presence of antibiotics may induce conjugation, although this effect may depend upon the antibiotic family (58). Plasmid transfer increases with the metabolic activity of the cell, following an increase in ATP levels (1). However, the effect of selection introduces a substantial bias, such that eliminating this variable, the presumed increase in conjugation almost disappears (86). Sub-inhibitory concentrations of biocides, like chlorhexidine, digluconate, or triclosan, and quaternary compounds, can increase in-vitro horizontal transfer events (40, 83).

Peracetic acid and sodium hypochlorite slightly increased conjugation rates (5). Additionally, non-antibiotic drugs that act on the central nervous system, such as carbamazepine, increase conjugation rates (140). Moreover, antipyretics like ibuprofen, naproxen, and gemfibrozil promote

364 the transmission of multidrug resistance plasmids at clinically and environmentally relevant
365 concentrations (39). Similar effects have been observed with artificial sweeteners and food
366 preservatives (2). Pesticides, as the insecticide cyromazine and the fungicide kresoxim-methyl,
367 greatly facilitate the conjugative transfer of antibiotic-resistance plasmids (148). Lastly, the
368 widespread use of herbicides also promotes AbR in soil microbiomes (81).

369 **2.11. Antimicrobials from phytoplankton, plants, and animals**

370 Phycotoxins are secondary metabolites produced primarily by photosynthetic micro- and
371 macroalgae in aquatic environments, including flagellates, diatoms, haptophytes, raphidophytes,
372 and cyanobacteria, and possess antibacterial properties (122). Phytotoxins, produced by many
373 plant species, including polyketides, peptides, and terpenes, also exhibit antibacterial activity and
374 eventually antibiofilm properties (73), and are utilized in food preservation and processing**.**
375 However, we lack systematic scientific knowledge about the antimicrobial mechanisms of
376 phytotoxins and their influence in the microbiosphere, as well as the potential of phyco- and
377 phytotoxins to influence microbial soil and water populations. Even less is known about the
378 mechanisms of bacterial resistance to these compounds and their potential for cross-resistance with
379 conventional antibiotics. However, for instance, the phytotoxin albicidin, inhibiting DNA gyrase,
380 has some cross-resistance to quinolones (36, 56).

381 Animals, ranging from insects to nematodes, reptiles, and mammals, possess antibacterial
382 zootoxins. Most correspond to innate immunity and are primarily based on antimicrobial peptides
383 that disrupt bacterial membranes, including cecropins, magainins, and defensins. Exposure to these
384 peptides can lead to mutations in genes encoding enzymes, transporters, transcriptional regulators,
385 and other essential bacterial functions, ultimately resulting in resistance to different classes of
386 antibiotics (25). Most animals produce substances that harm bacteria, which are ecologically linked

to the animal's anti-infective functions or its physiological microbiome. These substances include antimicrobials produced by commensal bacteria, such as antimetabolites and bacteriocins, which can be short peptides, like microcins (13) or larger proteins, like colicins.

2.12. Abundance and diversity of local bacterial populations

Cell density reduces the antibacterial effect of antibiotics by either decreasing the effective concentration of the antibiotic or lowering the per-cell antibiotic concentration (127). The collective of all human and animal-associated microbiotas forms a segmented yet stable and inheritable ecological reservoir, serving as the primary source of major antibiotic-resistant pathogenic and commensal bacteria, with high-density populations. These populations contaminate the external environment, particularly in areas with high humidity, optimal growth temperatures, a granular structure, and availability of natural nutrients. Consequently, the abundance and population density of bacteria indirectly influence nutrient availability; this relationship is non-linear, as higher abundance promotes greater population density through the excretion of chemical attractants, such as glycine. Regulatory mechanisms help alleviate the negative impact of excessive abundance; for example, if warm temperatures saturate the potential population size, individual bacteria may become cold-seeking (44).

Most bacteria in human microbiomes are commensals or opportunistic pathogens. Their dominance likely arises from an ancient evolutionary interaction with the host, leading to a greater tolerance for the host's antibacterial molecules. Consequently, human or animal commensals encounter therapeutic antimicrobials much more frequently than the minority, more transient pathogenic organisms, thus playing a critical role in spreading resistance genes (46). It is important to note that many antibiotic-resistance genes are not inherently designed to promote resistance but rather to perform physiological functions or resist other types of stress (16). Therefore, highly

complex external environments characterized by high microbial diversity and low antibiotic pressure also contain a wealth of antibiotic-resistance genes that can be acquired by humans.

The question of species diversity is also of critical importance; the higher the diversity, the less the probability of minoritarian (potentially pathogenic) populations acquiring resistance. First, due to the potential competition between predominant non-pathogenic commensals and pathogens for acting as recipients of mobile genetic elements. Second, the population of pathogens can be reduced by interspecies interactions involving antagonistic-amensalistic substances (98). Third, if it is true that HGT is facilitated by the close physical proximity of bacteria, the opposite phenomenon also occurs when the populations are extremely dense. Under these conditions, not only are bacterial antagonistic substances more effective, but conjugation becomes limited by the engagement time, the interval required between two successful matings (114).

If epidemics of resistance to pathogens occur frequently in hospitals, it is primarily because the antibiotics use significantly reduces microbiota diversity, allowing originally pathogenic minorities to dominate in number. This dominance facilitates the transfer of ARGs and mutational resistance (29, 61). Additionally, in dense populations, some mechanisms of resistance protect surrounding bacterial cells, promoting HGT (133).

2. EVOLUTIONARY DYNAMICS

Bacterial organisms often face unfavorable environmental conditions, displacing them from their optimal niches, where they can experience optimal reproduction or persistence, provoking a physiological adaptive stress or shock response. Classically, we differentiate conditions as starvation stress, osmotic stress, acid shock, cold or heat shock, oxidative DNA damage, or antimicrobial stress, many of them implying common sources of stress, such as envelope stress.

However, the response to all these types of stress is modulated by complex adaptive networks, typically controlled by two-component systems, including histidine kinases, transcriptional regulators, and sigma factors of RNA polymerase. Bacterial stress response is frequently the result of cross-talk among these systems, so it is tempting to propose that bacteria are unaware of the stress's origin, only of the consequences, the reduction in fitness. Therefore, there is a basic general stress response that can be exerted in different ways. A frequent adaptation mode is entering a low-energy style of life, such as the stationary phase, persistence, or sporulation. During the process of stress, the EcoK, as well as the EvoD of the population, reflected, for instance, by the number of substitution mutations, are deeply influenced (29). Evolutionary trends occur even during the stress-promoted stationary phase (150). On the other hand, different levels of stress intensity or combinations of different stresses can select different types of mutations or adaptive responses. If essentially there is a common global stress response, that does not preclude a certain imperfect specificity in some cases (38).

Evolvability involves not only inheritable variation established by specific selection but also the processes related to phenotypic variation. The concept of phenotypic variation originated in developmental biology, emphasizing the unity of the organism rather than a single trait as the primary driver of adaptation. New stochastic mutations, once incorporated into the genome, may create developmental constraints that shape the future direction of phenotypic evolution. This collection of mutations altering the antibiotic susceptibility phenotype has been labeled the “dark matter” or resistance (62). Any mutation, even one that does not provide a specific phenotype, may potentially alter a network of physiological traits, which can be analyzed by constructing a genetic covariance matrix, commonly referred to as the G matrix. The set of traits that will impart the highest fitness is the gmax, which may predict the optimum phenotypic adaptation (99, 131).

Consequently, the susceptibility phenotype to antibiotic action, resulting from cellular mechanisms, may vary under different environmental conditions. In summary, there is a wealth of variation in AbR in nature, independent of its clinical implications (67).

3.1. Temperature

Temperature influences the dynamics of bacterial cellular processes in various ways, and consequently, the dynamics of antibiotics and AbR. In *E. coli*, high temperatures decrease negative DNA supercoiling, increase protein misfolding, and promote protein aggregation, resulting in increased membrane fluidity and general membrane damage. Warm temperatures favor resistance to aminoglycosides, nitrofurantoin, or trimethoprim, likely influencing uptake modulation (87). Sub-lethal heat shock enhances the bactericidal effect of aminoglycosides, likely by promoting antibiotic uptake, increasing protein aggregation, and increasing reactive oxygen species (ROS) production (88).

Cold temperatures increase negative DNA supercoiling, reduce ribosomal translation processes, decrease membrane fluidity, alter fatty acid structural composition, and result in a general reduction in enzymatic activity. Low temperatures favor resistance to tetracyclines (97), macrolides, lincosamides, and fluoroquinolones (49). Similar phenotypes occur for warm-adapted and cold-adapted variants (38). In long-term evolution experiments (2,000 generations), the super-optimal temperature (42 °C) of *E. coli* populations increased the rate of substitution mutations by 1,000 times compared to the control (24, 31).

3.2. Acidity

The reasons explaining the vast genetic diversification of beta-lactamases remain poorly understood and cannot be solely attributed to exposure to a limited number of beta-lactam families.

A possible explanation is that specific mutations optimize the enzymatic effect under different environmental conditions, such as changes in local acidity influencing the dynamics of beta-lactamase molecules on the cellular external surface (50, 100). Acidity, including urine acidity, with a pH of 5, reduces fosfomycin chromosomal resistance but increases ciprofloxacin resistance in low-level resistant mutants (94). Additionally, acidity promotes AbR through plasmid conjugation (144).

3.3. Osmolarity

Osmolarity is increased in saline environments, as well as in soil and feces. In the presence of beta-lactams, the hydrostatic intracellular turgor pressure, which is otherwise essential for bacterial replication, promotes the mechanical rupture of the cytoplasmic membrane, followed by bacterial lysis. Similarly, aminoglycosides, either directly or indirectly, weaken the cellular membrane and lyses bacteria (138). Increasing osmolarity counteracts the intracellular turgor and protects bacteria from lysis (31, 113). Similar results have been observed for glycopeptides, and it is expected to occur for fosfomycin as well. Natural or artificial seawater tends to increase the minimum inhibitory concentration (MIC) of gentamicin, amikacin, nalidixic acid, tetracycline, and ciprofloxacin (91). In other organisms, such as *Klebsiella pneumoniae*, increased osmolarity represses efflux pumps, reducing resistance (132). Bacterial responses to hyperosmolarity and desiccation differ, but there are commonalities.

3.4. Oxygen availability

In *E. coli*, a reduction in dissolved oxygen levels (for instance, a decrease in aeration rate) impairs the rate of cell growth but promotes β-lactamase synthesis (116). Under anaerobic conditions, the ARGs expression is altered, leading to a significant decrease in resistance. For example, decreases

in resistance were observed for ertapenem (*bla*VIM ß-lactamases) and fosfomycin (*fos* genes). However, other genes were likely hyper-expressed, as evidenced by the *bla*OXA-48 carbapenemase (102). Fosfomycin chromosomal resistance mutations are also significantly less effective under anaerobic conditions, including those found in urine (94). In nature, oxygen deficiency is certainly intertwined with other ecological factors; for instance, in natural water bodies, the amount of dissolved oxygen depends on the depth of the water column, the action of winds and waves, the presence of photosynthetic organisms, temperature, and salinity (102).

3.5. Biofilms

The biofilm lifestyle, on its own, does not significantly increase antibiotic resistance (AbR) by raising the mutation rate. However, differential gene expression may occur. The expression of antibiotic efflux pumps is decreased in *P. aeruginosa* biofilms, thus not contributing significantly to AbR in these environments (42).

3.6. Nutrients

Nutrient deprivation of different molecules prevents cell envelope modifications due to its high energy demand, enhancing the effect of targeting antibiotics, e.g., polymyxins and β-lactams; this occurs even at concentrations that do not alter growth rate (76 The abundance of nutrients may also alter the susceptibility phenotype. For instance, mecillinam reversion to susceptibility varies between urine samples collected from different individuals, depending on cysteine levels, particularly under conditions of low osmolality (126). Metabolic genes, including those involved in cell stress response, are not unusual in conjugative plasmids; interestingly, they can contribute to AbR (103). Overall, metabolomics is emerging as a promising field of study in the expression of AbR phenotypes. The plasticity of metabolic routes under different environmental conditions

should influence those altered by antibiotic action, offering a wealth of non-genetic mutational phenotypes.

3.7. Radiation

In a phenomenon likely linked to oxidative stress, sunlight, particularly UV light, increases AbR, by increasing the mutation rate. It has been suggested that antibiotic gene expression is also triggered by radiation (34, 149). High-frequency electromagnetic waves reduce AbR (59). Radiation influences metal acquisition, as iron, which also affects oxidative stress and may increase the expression of resistance (84).

3.8. Antimicrobial agents, pharmaceuticals, mycotoxins

Stress induced by exposure to antimicrobial agents influences the mutation rate, primarily due to the effect of superoxide released during the SOS stress response, which damages bacterial DNA (14). This phenomenon was first discovered for fluoroquinolones, which also damage DNA, ultimately facilitating AbR (28). However, antibiotic exposure may increase the number of mutations through mechanisms independent of the induced SOS response, as seen with the hyperexpression of dinB, which encodes the error-prone DNA polymerase IV (27). Some microorganisms excrete toxins, such as cytosine deaminases, that have mutagenic activity on bacteria, ultimately contributing to the emergence of antibiotic-resistant phenotypes (43). In the environment, mycotoxins—numerous antibiotic-like molecules—can act on bacteria, potentially increasing the mutation rate in stressed cells (77). However, mutations leading to resistance are not the only consequence of antibiotic exposure at subinhibitory concentrations; another possibility is the selection of persisters, a subpopulation of cells that can survive intensive antibiotic treatment without developing resistance. In this trade-off, there is an increase in the emergence of mutations

and a decrease in the production of persister cells, thereby reducing persistence levels (65). Not primarily antimicrobial compounds, including antidepressants, increase AbR following superoxide mutagenesis (68). This suggests that the coexistence of antidepressants and antibiotics, either in individuals or in the environment, might occur and impact AbR.

3.9. Host Colonization and Infection

The colonization of host internal and external surfaces, including in humans, implies bacterial adaptation to host molecules that may provoke bacterial stress. In some cases, it can be expected that the biological cost associated with the expression of resistance mechanisms may be increased by antibacterial substances, thus explaining the persistence over time of antibiotic-susceptible populations. For instance, intestinal colonization generates bacterial stress derived from acidity, biliary acids, pancreatic enzymes, short-, medium-, and long-chain fatty acids, polyphenols, flavonoids, polyamines, host, and bacterial antimicrobial peptides (20).

The microecological conditions that bacteria encounter during the infection should influence the evolutionary dynamics of AbR. Tissue and intracellular invasion, as well as phagocytosis, exert a strong stress on bacterial cells. Antimicrobial peptides from innate immunity can be mutagenic, as well as superoxide exposure in phagosomes. Phenotypic phase-like variation altering antibiotic susceptibility occurs in phagocytes merely as a consequence of the more acidic conditions (30). Infection, mutagenesis, virulence, and AbR are frequently linked characters, which can explain the expansion and genetic diversification of bacterial clones during epidemics (93, 117).

3. THE INTERPLAY of EcoK-EvoD IN ECOLOGICAL COMPLEX LANDSCAPES AND THE RISK ASSESSMENT FOR ANTIBIOTIC RESISTANCE

Ecological kinetics constantly trade off with evolutionary dynamics. Ecological changes expose bacterial organisms to various stressors. Chemotaxis is an EcoK strategy used to locate areas where ecological conditions minimize nutritional environmental stress. Chemoattractants include amino acids, organic acids, fatty acids, sugars, polyamines, quaternary amines, purines, pyrimidines, aromatic hydrocarbons, oxygen, inorganic ions, and polysaccharides, resulting in changes to the ecological distribution of microorganisms (95). Antibiotic stress from environmental aminoglycosides or tetracyclines can be mitigated in chemotactic *E. coli* as they migrate to more acidic environments, where the dynamic effects of these antibiotics are weakened (141). However, escaping one type of stress may expose the population to new stressful conditions, and this way out of the vicious circle requires EvoD. This explains the constant emergence of numerous random adaptive mutations, including those derived from transposition events triggered by stress, many of which directly or indirectly influence AbR (67, 85).

Unfortunately, our understanding of the landscape ecology and quantitative biology of resistant pathogens, which informs us about the absolute abundance of specific resistant species or clones in various environments at specific times, remains quite limited (19). A crucial point to consider is the challenge of obtaining reliable data for general application; microbial ecology is inherently local, primarily based in microecological environments that are highly diverse and variable. Of course, our role as biological scientists is to confront this complexity to identify the conditions, particularly the combinations of conditions, that might pose a specific risk for the dispersal (EcoK), emergence, and/or evolution (EvoD) of AbR, both in organisms and genes. The concatenated

figures 1-4 aim to provide the reader with a schema for identifying local risk areas and can serve as a conceptual frame to illustrate how this challenge can be addressed.

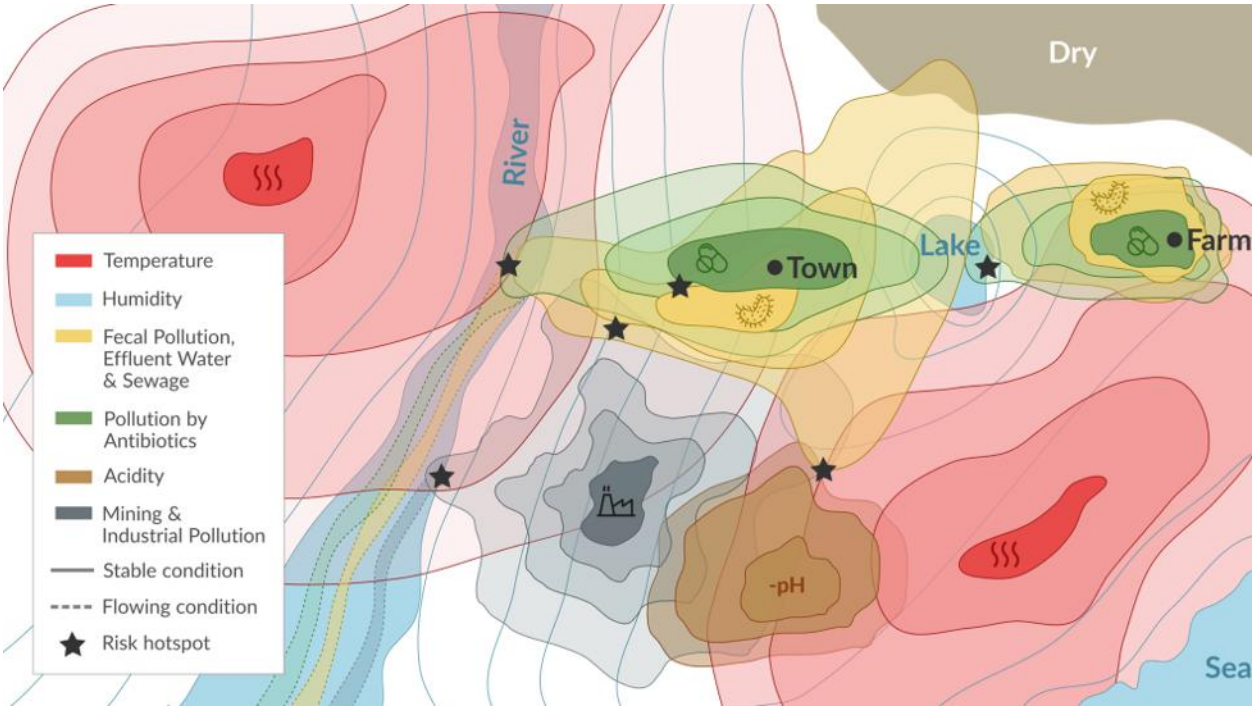

Figure 1. A schematic chart illustrating the various natural and artificial conditions that may influence the regional abundance and transmission of antibiotic resistance. These conditions, represented in different colors, vary spatially, resulting in multiple gradients emanating from their sources. The overlapping of these gradients forms distinct spaces or patches (arbitrary black stars) where several conditions impact the anti-biotic resistance load.

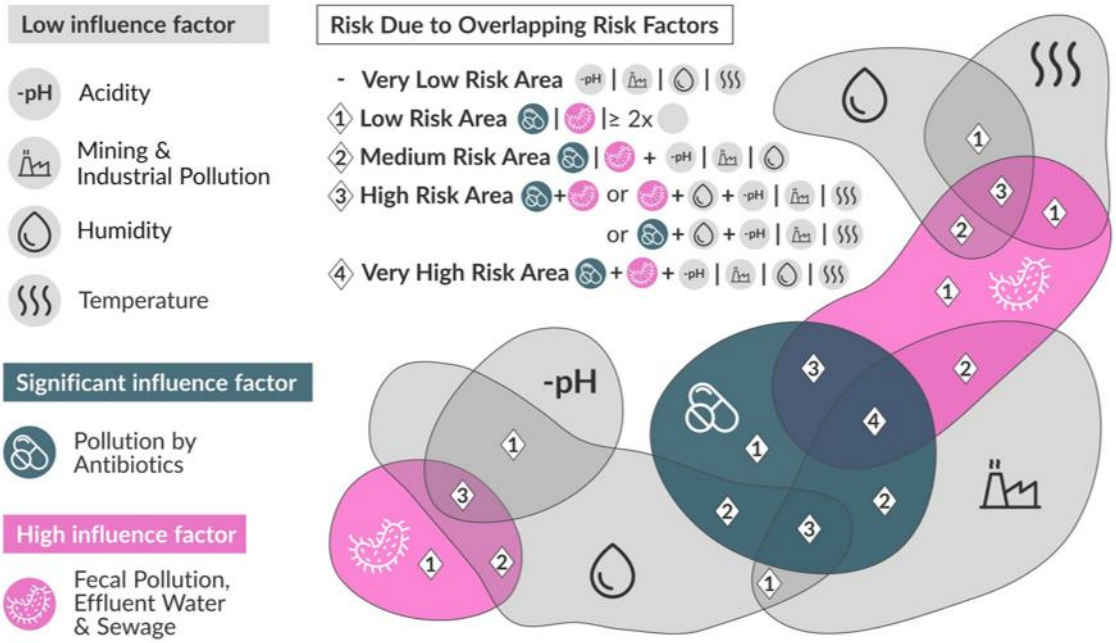

Figure 2. Conceptual schema showing how overlapping ecological conditions may determine the risks of antibiotic resistance. In this simplified version of an ecological landscape (as in Figure 1), different drivers of selection for antibiotic resistance are represented in different tones, with darker ones indicating higher risks. In the presence of fecal pollution, there is a significant risk of resistance; however, if the conditions are not favorable for high bacterial density or selection, the risk is low (1). A certain, but low risk (1), also occurs in areas facilitating a high density of environmental bacteria. The intersection of fecal contamination and antibiotic exposure creates a high-risk area (3), and industrial pollution further exacerbates this risk (4). However, even in the absence of fecal bacteria, a determining antibiotic contamination can still select for antibiotic resistance in environmental bacteria, particularly in the presence of industrial pollution and high humidity, thereby facilitating growth and spread, and in the presence of stress, as low pH (2). A similar risk arises in the absence of antibiotics when fecal bacteria are exposed to environmental stressors, such as high temperatures, acidity, and favorable growth conditions, including high humidity (2). This schema illustrates the multiplicity and co-determination of environmental factors involved in ecological dynamics, including the growth and spread of resistant bacteria, as well as evolutionary kinetics, which lead to the emergence and selection of resistant organisms. The schema emphasized the need to measure local environmental conditions of different ecological patches to determine surveillance areas for antibiotic resistance, but the currently available methods and data to quantify the local risks remain extremely limited.

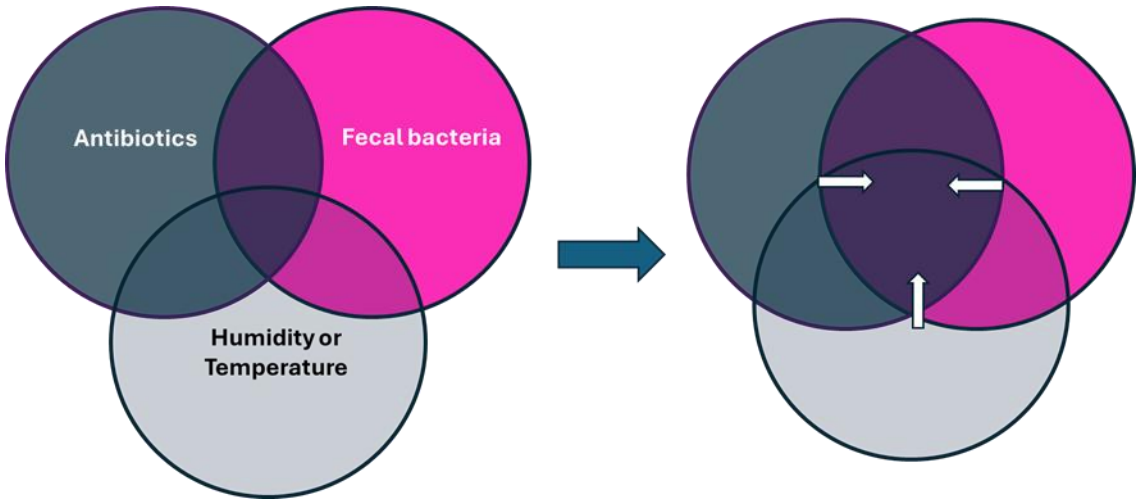

Figure 3. **The overlapping ecological conditions that determine the size of risk areas for antibiotic resistance change over time**. This schema illustrates how the darker, high-risk areas are enlarged when the circles representing antibiotic pollution, fecal bacteria pollution, and other factors that facilitate bacterial growth, such as humidity or temperature, converge (white arrows) during specific periods of time.

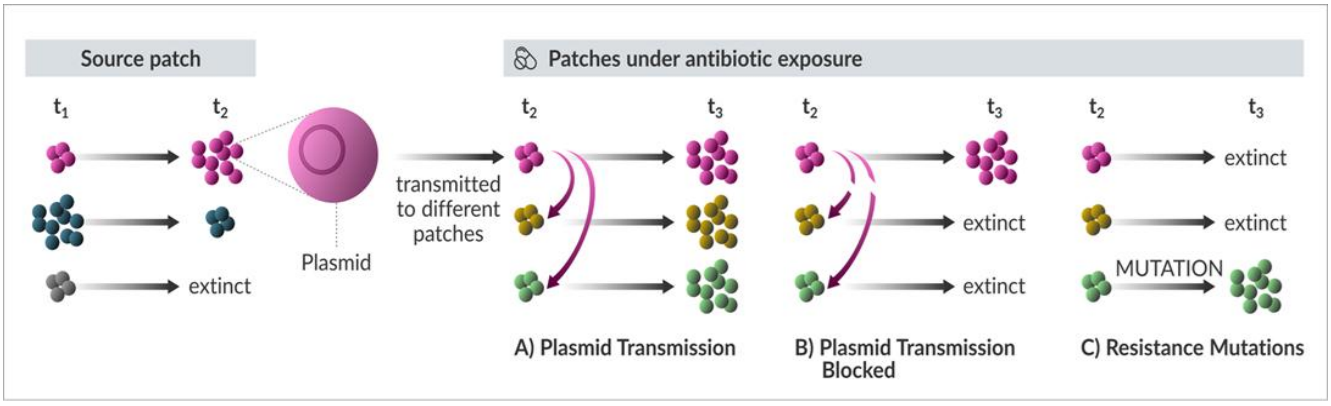

Figure 4. The ecological conditions of different areas or patches determine the ecological dynamics and evolutionary kinetics of antibiotic resistance. Variation in ecological conditions from time t1 to t2 in various source environmental patches may lead to an increase, decrease, or even provocation of extinction for certain organisms. On the right side, under antibiotic exposure, the pink species boosts its population because it carries a plasmid (red ring) with resistance genes. A: From this source patch, the organism can migrate (arrows) to other patches and transmit its plasmid to the local kin, yellow and green bacteria, thereby protecting them from extinction and allowing their populations to grow from time t2 to t3. B: However, the ecological conditions of the other patches may hinder plasmid transmission, so protection does not occur (broken arrows), and the bacterial population in these patches faces extinction. C: If the conditions of the patch prevent protection by the plasmid, the red and yellow populations face extinction, but these conditions may increase mutagenesis in the green population, which survives and grows in population size.

These trade-offs in conditions that affect AbR phenotypes occur at the intersection of gradients; each concentration in a gradient may serve as a specific selector for a particular bacterial variation, known as concentration-specific selection (18). This phenomenon significantly adds to the complexity of AbR. However, we are rapidly making progress in technological advancements toward networks of sensors that gather environmental data, which are then integrated to predict the probability of hotspots for AbR, including metagenomic data analysis (41, 64, 105). Eco-evolutionary response modeling and modern cellular computational models can assist in such tasks (29, 104). Additionally, advancements in Artificial Intelligence and Machine Learning offer exciting prospects for condensing relevant information to enhance the EcoK-EvoD knowledge of AbR (107). Our main conclusion is that AbR emerges, is selected, spreads, and is expressed due to a vast multiplicity, a connected universe of ecological drivers of various natures, particularly, but not necessarily (75), acting in combination with antibiotic exposure. Identifying those areas that condense a specific combination of conditions at a given time, favoring AbR, determines the hotspots for monitoring AbR processes (23).

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LITERATURE CITED

1. Aduru SV, Szenkiel K, Rahman A, Ahmad M, Fabozzi M, et al. 2024. Sub-inhibitory antibiotic treatment selects for enhanced metabolic efficiency. *Microbiol. Spectr.* 12(2):e0324123
2. Alav I, Buckner MMC. 2024. Non-antibiotic compounds associated with humans and the environment can promote horizontal transfer of antimicrobial resistance genes. *Crit. Rev. Microbiol.* 50(6):993–1010
3. Almblad H, Randall TE, Liu F, Leblanc K, Groves RA, et al. 2021. Bacterial cyclic diguanylate signaling networks sense temperature. *Nat. Commun.* 12(1):1986
4. Alsaffar L, Osborne L, Bourne NT. 2018. Bacterial colonization of bladeless electrical fans. *J. Hosp. Infect.* 100(4):476–77
5. Alvarez-Molina A, Trigal E, Prieto M, López M, Alvarez-Ordóñez A. 2023. Assessment of a plasmid conjugation procedure to monitor horizontal transfer of an extended-spectrum β-

lactamase resistance gene under food chain scenarios. *Curr. Res. Food Sci.* 6:100405

6. Anbalagan S. 2024. Gas-sensing riboceptors. *RNA Biol.* 21(1):1–6
7. APS -2024 APS March Meeting - Event - Does hyperosmotic stress reduce bacterial growth rate by decreasing enzymatic velocity?
8. Aracil-Gisbert S, Fernández-De-Bobadilla MD, Guerra-Pinto N, Serrano-Calleja S, Pérez-Cobas AE, et al. The ICU environment contributes to the endemicity of the "Serratia marcescens complex" in the hospital setting. *mBio*. 15(5):e03054-23
9. *Are Sewage Treatment Plants Promoting Antibiotic Resistance?: Critical Reviews in Environmental Science and Technology: Vol 41, No 3*. https://www.tandfonline.com/doi/abs/10.1080/10643380902772589
10. Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. 2006. Co-selection of antibiotic and metal resistance. *Trends Microbiol.* 14(4):176–82
11. Baquero F, Beis K, Craik DJ, Li Y, Link AJ, et al. 2024. The pearl jubilee of microcin J25: thirty years of research on an exceptional lasso peptide. *Nat. Prod. Rep.* 41(3):469–511
12. Baquero F, Coque TM, Guerra-Pinto N, Galán JC, Jiménez-Lalana D, et al. 2022. The Influence of Coalescent Microbiotic Particles From Water and Soil on the Evolution and Spread of Antimicrobial Resistance. *Front. Environ. Sci.* 10:
13. Baquero F, Lanza VF, Baquero M-R, del Campo R, Bravo-Vázquez DA. 2019. Microcins in Enterobacteriaceae: Peptide Antimicrobials in the Eco-Active Intestinal Chemosphere. *Front. Microbiol.* 10:
14. Baquero F, Levin BR. 2021. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat. Rev. Microbiol.* 19(2):123–32
15. Baquero F, Martínez J-L, Cantón R. 2008. Antibiotics and antibiotic resistance in water

environments. *Curr. Opin. Biotechnol.* 19(3):260–65

16. Baquero F, Martínez JL, F Lanza V, Rodríguez-Beltrán J, Galán JC, et al. 2021. Evolutionary Pathways and Trajectories in Antibiotic Resistance. *Clin. Microbiol. Rev.* 34(4):e0005019
17. Baquero F, Martínez J-L, Sánchez A, Fernández-de-Bobadilla MD, San-Millán A, Rodríguez-Beltrán J. 2023. Bacterial Subcellular Architecture, Structural Epistasis, and Antibiotic Resistance. *Biology*. 12(5):640
18. Baquero F, Negri MC. 1997. Selective compartments for resistant microorganisms in antibiotic gradients. *BioEssays News Rev. Mol. Cell. Dev. Biol.* 19(8):731–36
19. Baquero F, Pérez-Cobas AE, Aracil-Gisbert S, Coque TM, Zamora J. 2024. Selection versus transmission: Quantitative and organismic biology in antibiotic resistance. *Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis.* 121:105606
20. Baquero F, Rodríguez-Beltrán J, Coque TM, Del Campo R. 2024. Boosting Fitness Costs Associated with Antibiotic Resistance in the Gut: On the Way to Biorestoration of Susceptible Populations. *Biomolecules*. 14(1):76
21. Beizman-Magen Y, Grinberg M, Orevi T, Kashtan N. 2022. Wet-dry cycles protect surface-colonizing bacteria from major antibiotic classes. *ISME J.* 16(1):91–100
22. Belykh E, Maystrenko T, Velegzhaninov I, Tavleeva M, Rasova E, Rybak A. 2024. Taxonomic Diversity and Functional Traits of Soil Bacterial Communities under Radioactive Contamination: A Review. *Microorganisms*. 12(4):733
23. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, et al. 2015. Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol.* 13(5):310–17
24. Billings G, Ouzounov N, Ursell T, Desmarais SM, Shaevitz J, et al. 2014. De novo

morphogenesis in L-forms via geometric control of cell growth. *Mol. Microbiol.* 93(5):883–96

25. Blanco P, Hjort K, Martínez JL, Andersson DI. 2020. Antimicrobial Peptide Exposure Selects for Resistant and Fit Stenotrophomonas maltophilia Mutants That Show Cross-Resistance to Antibiotics. *mSphere*. 5(5):e00717-20
26. Blankenhorn D, Phillips J, Slonczewski JL. 1999. Acid- and base-induced proteins during aerobic and anaerobic growth of Escherichia coli revealed by two-dimensional gel electrophoresis. *J. Bacteriol.* 181(7):2209–16
27. Blázquez J, Couce A, Rodríguez-Beltrán J, Rodríguez-Rojas A. 2012. Antimicrobials as promoters of genetic variation. *Curr. Opin. Microbiol.* 15(5):561–69
28. Bush NG, Diez-Santos I, Sankara Krishna P, Clavijo B, Maxwell A. 2025. Insights into antibiotic resistance promoted by quinolone exposure. *Antimicrob. Agents Chemother.* 69(1):e0099724
29. Campos M, Capilla R, Naya F, Futami R, Coque T, et al. 2019. Simulating Multilevel Dynamics of Antimicrobial Resistance in a Membrane Computing Model. *mBio*. 10(1):e02460-18
30. Castanheira S, López-Escarpa D, Pucciarelli MG, Cestero JJ, Baquero F, García-Del Portillo F. 2020. An alternative penicillin-binding protein involved in Salmonella relapses following ceftriaxone therapy. *EBioMedicine*. 55:102771
31. Cesar S, Anjur-Dietrich M, Yu B, Li E, Rojas E, et al. 2020. Bacterial Evolution in High-Osmolarity Environments. *mBio*. 11(4):e01191-20
32. Cesar S, Anjur-Dietrich M, Yu B, Li E, Rojas E, et al. 2020. Bacterial Evolution in High-Osmolarity Environments. *mBio*. 11(4):e01191-20

753 33. Chen X, Yin H, Li G, Wang W, Wong PK, et al. 2019. Antibiotic-resistance gene transfer in
754 antibiotic-resistance bacteria under different light irradiation: Implications from oxidative
755 stress and gene expression. *Water Res.* 149:282–91
756 34. Chen X, Yin H, Li G, Wang W, Wong PK, et al. 2019. Antibiotic-resistance gene transfer in
757 antibiotic-resistance bacteria under different light irradiation: Implications from oxidative
758 stress and gene expression. *Water Res.* 149:282–91
759 35. Chevalier C, Stojanović O, Colin DJ, Suarez-Zamorano N, Tarallo V, et al. 2015. Gut
760 Microbiota Orchestrates Energy Homeostasis during Cold. *Cell*. 163(6):1360–74
761 36. *Comparison of Strategies to Overcome Drug Resistance: Learning from Various Kingdoms*.
762 https://www.mdpi.com/1420-3049/23/6/1476
763 37. Corkrey R, McMeekin TA, Bowman JP, Ratkowsky DA, Olley J, Ross T. 2016. The
764 Biokinetic Spectrum for Temperature. *PloS One*. 11(4):e0153343
765 38. Cruz-Loya M, Kang TM, Lozano NA, Watanabe R, Tekin E, et al. 2019. Stressor interaction
766 networks suggest antibiotic resistance co-opted from stress responses to temperature. *ISME*
767 *J.* 13(1):12–23
768 39. Cui Y, Gao J, Guo Y, Li Z, Wang Z, Zhao Y. 2022. Unraveling the impact and mechanism
769 of antipyretic paracetamol on intergenera conjugative plasmid transfer. *Environ. Res.* 215(Pt
770 1):114263
771 40. Curiao T, Marchi E, Grandgirard D, León-Sampedro R, Viti C, et al. 2016. Multiple adaptive
772 routes of Salmonella enterica Typhimurium to biocide and antibiotic exposure. *BMC*
773 *Genomics*. 17:491
774 41. Davis BC, Vikesland PJ, Pruden A. 2025. Evaluating Quantitative Metagenomics for
775 Environmental Monitoring of Antibiotic Resistance and Establishing Detection Limits.

776 *Environ. Sci. Technol.* 59(12):6192–6202
777 42. De Kievit TR, Parkins MD, Gillis RJ, Srikumar R, Ceri H, et al. 2001. Multidrug Efflux
778 Pumps: Expression Patterns and Contribution to Antibiotic Resistance in Pseudomonas
779 aeruginosa Biofilms. *Antimicrob. Agents Chemother.* 45(6):1761–70
780 43. de Moraes MH, Hsu F, Huang D, Bosch DE, Zeng J, et al. An interbacterial DNA deaminase
781 toxin directly mutagenizes surviving target populations. *eLife*. 10:e62967
782 44. Demir M, Douarche C, Yoney A, Libchaber A, Salman H. 2011. Effects of population
783 density and chemical environment on the behavior of Escherichia coli in shallow temperature
784 gradients. *Phys. Biol.* 8(6):063001
785 45. DeVilbiss SE, Steele MK, Krometis L-AH, Badgley BD. 2021. Freshwater salinization
786 increases survival of Escherichia coli and risk of bacterial impairment. *Water Res.*
787 191:116812
788 46. Dionisio F, Domingues CPF, Rebelo JS, Monteiro F, Nogueira T. 2023. The Impact of Non-
789 Pathogenic Bacteria on the Spread of Virulence and Resistance Genes. *Int. J. Mol. Sci.*
790 24(3):1967
791 47. Elser JJ, Sterner RW, Gorokhova E, Fagan WF, Markow TA, et al. 2000. Biological
792 stoichiometry from genes to ecosystems. *Ecol. Lett.* 3(6):540–50
793 48. Environment UN. 2023. *Bracing for Superbugs: Strengthening environmental action in the*
794 *One Health response to antimicrobial resistance | UNEP - UN Environment Programme*.
795 https://www.unep.org/resources/superbugs/environmental-action
796 49. Fàbrega A, Madurga S, Giralt E, Vila J. 2009. Mechanism of action of and resistance to
797 quinolones. *Microb. Biotechnol.* 2(1):40–61
798 50. Francisco JA, Earhart CF, Georgiou G. 1992. Transport and anchoring of beta-lactamase to

the external surface of Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* 89(7):2713–17

51. Furlan JPR, Sellera FP, Lincopan N, Debone D, Miraglia SGEK, Tavella RA. 2024. Catastrophic floods and antimicrobial resistance: Interconnected threats with wide-ranging impacts. *One Health Amst. Neth.* 19:100891
52. Fuzi M. 2025. The fitness connection of antibiotic resistance. *Front. Microbiol.* 16:1556656
53. García-Descalzo L, García-López E, Alcázar A, Baquero F, Cid C. 2014. Proteomic analysis of the adaptation to warming in the Antarctic bacteria Shewanella frigidimarina. *Biochim. Biophys. Acta*. 1844(12):2229–40
54. Ghigo JM. 2001. Natural conjugative plasmids induce bacterial biofilm development. *Nature*. 412(6845):442–45
55. Hancock AM, Dill-Macky AS, Moore JA, Day C, Donia MS, Datta SS. 2025. A nutrient bottleneck limits antibiotic efficacy in structured bacterial populations
56. Hashimi SM, Wall MK, Smith AB, Maxwell A, Birch RG. 2007. The phytotoxin albicidin is a novel inhibitor of DNA gyrase. *Antimicrob. Agents Chemother.* 51(1):181–87
57. Hashimoto M, Hasegawa H, Maeda S. 2019. High temperatures promote cell-to-cell plasmid transformation in Escherichia coli. *Biochem. Biophys. Res. Commun.* 515(1):196–200
58. Headd B, Bradford SA. 2018. Physicochemical Factors That Favor Conjugation of an Antibiotic Resistant Plasmid in Non-growing Bacterial Cultures in the Absence and Presence of Antibiotics. *Front. Microbiol.* 9:2122
59. Hegazy EA, El-Antrawy MA. 2025. Impact of high frequency electromagnetic radiation on bacterial survival and antibiotic activity in exposed bacteria. *Sci. Rep.* 15(1):7852
60. Hoshino T, Doi H, Uramoto G-I, Wörmer L, Adhikari RR, et al. 2020. Global diversity of microbial communities in marine sediment. *Proc. Natl. Acad. Sci. U. S. A.* 117(44):27587–97

61. *https://edoctorado.ucm.es/file/listado-de-tesis-en-publicidad-de-17-03-2025-a-31-03-2025?ver*. https://edoctorado.ucm.es/file/listado-de-tesis-en-publicidad-de-17-03-2025-a-31-03-2025?ver
62. Hughes D, Andersson DI. 2017. Environmental and genetic modulation of the phenotypic expression of antibiotic resistance. *FEMS Microbiol. Rev.* 41(3):374–91
63. Huus KE, Ley RE. 2021. Blowing Hot and Cold: Body Temperature and the Microbiome. *mSystems*. 6(5):e0070721
64. Inobeme A, Natarajan A, Pradhan S, Adetunji CO, Ajai AI, et al. 2024. Chemical Sensor Technologies for Sustainable Development: Recent Advances, Classification, and Environmental Monitoring. *Adv. Sens. Res.* 3(12):2400066
65. Ismail AS, Berryhill BA, Gil-Gil T, Manuel JA, Smith AP, et al. 2024. The Tradeoffs Between Persistence and Mutation Rates at Sub-Inhibitory Antibiotic Concentrations in Staphylococcus aureus. *BioRxiv Prepr. Serv. Biol.* 2024.04.01.587561
66. Jadeja NB, Worrich A. 2022. From gut to mud: dissemination of antimicrobial resistance between animal and agricultural niches. *Environ. Microbiol.* 24(8):3290–3306
67. Jago MJ, Soley JK, Denisov S, Walsh CJ, Gifford DR, et al. 2025. High-throughput method characterizes hundreds of previously unknown antibiotic resistance mutations. *Nat. Commun.* 16(1):780
68. Jin M, Lu J, Chen Z, Nguyen SH, Mao L, et al. 2018. Antidepressant fluoxetine induces multiple antibiotics resistance in Escherichia coli via ROS-mediated mutagenesis. *Environ. Int.* 120:421–30
69. Jin Q, Kirk MF. 2018. pH as a Primary Control in Environmental Microbiology: 1. Thermodynamic Perspective. *Front. Environ. Sci.* 6:

70. Johnsen AR, Kroer N. 2007. Effects of stress and other environmental factors on horizontal plasmid transfer assessed by direct quantification of discrete transfer events. *FEMS Microbiol. Ecol.* 59(3):718–28
71. Johnsen AR, Kroer N. 2007. Effects of stress and other environmental factors on horizontal plasmid transfer assessed by direct quantification of discrete transfer events. *FEMS Microbiol. Ecol.* 59(3):718–28
72. Khalil TA, Gealt MA. 1987. Temperature, pH, and cations affect the ability of Escherichia coli to mobilize plasmids in L broth and synthetic wastewater. *Can. J. Microbiol.* 33(8):733–37
73. Khameneh B, Eskin NAM, Iranshahy M, Fazly Bazzaz BS. 2021. Phytochemicals: A Promising Weapon in the Arsenal against Antibiotic-Resistant Bacteria. *Antibiot. Basel Switz.* 10(9):1044
74. Klümper U, Dechesne A, Riber L, Brandt KK, Gülay A, et al. 2017. Metal stressors consistently modulate bacterial conjugal plasmid uptake potential in a phylogenetically conserved manner. *ISME J.* 11(1):152–65
75. Knöppel A, Näsvall J, Andersson DI. 2017. Evolution of Antibiotic Resistance without Antibiotic Exposure. *Antimicrob. Agents Chemother.* 61(11):e01495-17
76. Kok M, Hankemeier T, van Hasselt JGC. 2025. Nutrient conditions affect antimicrobial pharmacodynamics in Pseudomonas aeruginosa. *Microbiol. Spectr.* 13(1):e0140924
77. Kothapalli PK, Nair MNB, Punniyamurthy N. 2021. MYCOTOXIN INDUCED ANTIBIOTIC RESISTANCE, AN UNSUSPECTED PUBLIC HEALTH IMPACT: A PERSPECTIVE REVIEW. *Indian J. Vet. Anim. Sci. Res.* 50(4):1–15
78. Larsson DGJ, Flach C-F. 2022. Antibiotic resistance in the environment. *Nat. Rev.*

Microbiol. 20(5):257–69

79. Lenski RE, Barrick JE, Ofria C. 2006. Balancing Robustness and Evolvability. *PLoS Biol.* 4(12):e428

80. Li S, Bai Y, Li Z, Wang A, Ren N-Q, Ho S-H. 2025. Overlooked role of extracellular polymeric substances in antibiotic-resistance gene transfer within microalgae-bacteria system. *J. Hazard. Mater.* 488:137206

81. Liao H, Li X, Yang Q, Bai Y, Cui P, et al. 2021. Herbicide Selection Promotes Antibiotic Resistance in Soil Microbiomes. *Mol. Biol. Evol.* 38(6):2337–50

82. Lin H, Wang D, Wang Q, Mao J, Yang L, et al. 2025. Epigenetic modifications and metabolic gene mutations drive resistance evolution in response to stimulatory antibiotics. *Mol. Syst. Biol.* 21(3):294–314

83. Liu C, Goh SG, You L, Yuan Q, Mohapatra S, et al. 2023. Low concentration quaternary ammonium compounds promoted antibiotic resistance gene transfer via plasmid conjugation. *Sci. Total Environ.* 887:163781

84. Liu M, Wang M, Huang M, Gao Q, Zhu D, et al. 2024. Iron efflux by IetA enhances β-lactam aztreonam resistance and is linked to oxidative stress through cellular respiration in Riemerella anatipestifer. *J. Antimicrob. Chemother.* 79(6):1385–96

85. Lopatkin AJ, Bening SC, Manson AL, Stokes JM, Kohanski MA, et al. 2021. Clinically relevant mutations in core metabolic genes confer antibiotic resistance. *Science*. 371(6531):eaba0862

86. Lopatkin AJ, Huang S, Smith RP, Srimani JK, Sysoeva TA, et al. 2016. Antibiotics as a selective driver for conjugation dynamics. *Nat. Microbiol.* 1(6):16044

87. Loughman K, Hall J, Knowlton S, Sindeldecker D, Gilson T, et al. 2016. Temperature-

Dependent Gentamicin Resistance of Francisella tularensis is Mediated by Uptake Modulation. *Front. Microbiol.* 7:37

88. Lv B, Huang X, Lijia C, Ma Y, Bian M, et al. 2023. Heat shock potentiates aminoglycosides against gram-negative bacteria by enhancing antibiotic uptake, protein aggregation, and ROS. *Proc. Natl. Acad. Sci. U. S. A.* 120(12):e2217254120
89. Lv B, Jiang C, Han Y, Wu D, Jin L, et al. 2024. Diverse bacterial hosts and potential risk of antibiotic resistomes in ship ballast water revealed by metagenomic binning. *Environ. Res.* 253:119056
90. MacFadden DR, McGough SF, Fisman D, Santillana M, Brownstein JS. 2018. Antibiotic Resistance Increases with Local Temperature. *Nat. Clim. Change*. 8(6):510–14
91. Magalhães RMR. Study of the influence of seawater in antibiotic susceptibility
92. Manaia CM, Aga DS, Cytryn E, Gaze WH, Graham DW, et al. 2024. The Complex Interplay Between Antibiotic Resistance and Pharmaceutical and Personal Care Products in the Environment. *Environ. Toxicol. Chem.* 43(3):637–52
93. Martínez JL, Baquero F. 2002. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin. Microbiol. Rev.* 15(4):647–79
94. Martín-Gutiérrez G, Docobo-Pérez F, Rodriguez-Beltrán J, Rodríguez-Martínez JM, Aznar J, et al. 2017. Urinary Tract Conditions Affect Fosfomycin Activity against Escherichia coli Strains Harboring Chromosomal Mutations Involved in Fosfomycin Uptake. *Antimicrob. Agents Chemother.* 62(1):e01899-17
95. Matilla MA, Krell T. 2024. Bacterial amino acid chemotaxis: a widespread strategy with multiple physiological and ecological roles. *J. Bacteriol.* 206(10):e0030024

96. McMeekin TA, Olley J, Ratkowsky DA. 1988. Temperature Effects on Bacterial Growth Rates. In *Physiological Models in Microbiology*. CRC Press. 15 pp.
97. Miller JH, Novak JT, Knocke WR, Pruden A. 2014. Elevation of antibiotic resistance genes at cold temperatures: implications for winter storage of sludge and biosolids. *Lett. Appl. Microbiol.* 59(6):587–93
98. Nair RR, Andersson DI. 2023. Interspecies interaction reduces selection for antibiotic resistance in Escherichia coli. *Commun. Biol.* 6(1):1–9
99. Nei M. 2007. The new mutation theory of phenotypic evolution. *Proc. Natl. Acad. Sci. U. S. A.* 104(30):12235–42
100. Ohsuka S, Arakawa Y, Horii T, Ito H, Ohta M. 1995. Effect of pH on activities of novel beta-lactamases and beta-lactamase inhibitors against these beta-lactamases. *Antimicrob. Agents Chemother.* 39(8):1856–58
101. Oliveira NM, Martinez-Garcia E, Xavier J, Durham WM, Kolter R, et al. 2015. Biofilm Formation As a Response to Ecological Competition. *PLoS Biol.* 13(7):e1002191
102. Ortiz-Miravalles L, Prieto A, Kieffer N, Vergara E, Cantón R, et al. 2025. Effect of oxygen on antimicrobial resistance genes from a one health perspective. *Sci. Total Environ.* 979:179523
103. Palomino A, Gewurz D, DeVine L, Zajmi U, Moralez J, et al. 2023. Metabolic genes on conjugative plasmids are highly prevalent in Escherichia coli and can protect against antibiotic treatment. *ISME J.* 17(1):151–62
104. Pinheiro F. 2024. Predicting the evolution of antibiotic resistance. *Curr. Opin. Microbiol.* 82:102542
105. Pratap AR, Annamalai S. 2025. Sensor Technologies for Environmental Data Collection.

In *Environmental Monitoring Using Artificial Intelligence*, ed A Suresh, T Devi, N Deepa, AK Bashir, pp. 133–66. Wiley. 1st ed.

106. Qiu Y, Zhou Y, Chang Y, Liang X, Zhang H, et al. 2022. The Effects of Ventilation, Humidity, and Temperature on Bacterial Growth and Bacterial Genera Distribution. *Int. J. Environ. Res. Public. Health*. 19(22):15345

107. Rafiq K, Beery S, Palmer MS, Harchaoui Z, Abrahms B. 2025. Generative AI as a tool to accelerate the field of ecology. *Nat. Ecol. Evol.* 9(3):378–85

108. Ramakrishnan B, Venkateswarlu K, Sethunathan N, Megharaj M. 2019. Local applications but global implications: Can pesticides drive microorganisms to develop antimicrobial resistance? *Sci. Total Environ.* 654:177–89

109. Raro OHF, Poirel L, Nordmann P. 2023. Effect of Zinc Oxide and Copper Sulfate on Antibiotic Resistance Plasmid Transfer in Escherichia coli. *Microorganisms*. 11(12):2880

110. Reinthaler FF, Posch J, Feierl G, Wüst G, Haas D, et al. 2003. Antibiotic resistance of E. coli in sewage and sludge. *Water Res.* 37(8):1685–90

111. Reisner A, Höller BM, Molin S, Zechner EL. 2006. Synergistic Effects in Mixed Escherichia coli Biofilms: Conjugative Plasmid Transfer Drives Biofilm Expansion. *J. Bacteriol.* 188(10):3582–88

112. REX Consortium. 2013. Heterogeneity of selection and the evolution of resistance. *Trends Ecol. Evol.* 28(2):110–18

113. Rodriguez MB, Moysés LHC, Costa SOP. 1990. Effect of osmolarity on aminoglycoside susceptibility in Gram-negative bacteria. *Lett. Appl. Microbiol.* 11(2):77–80

114. Rodriguez-Grande J, Ortiz Y, Garcia-Lopez D, Garcillán-Barcia MP, de la Cruz F, Fernandez-Lopez R. 2025. Encounter rates and engagement times limit the transmission of

conjugative plasmids. *PLoS Genet.* 21(2):e1011560

115. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, et al. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4(10):1340–51

116. Ryan W, Parulekar SJ, Stark BC. 1989. Expression of beta-lactamase by recombinant Escherichia coli strains containing plasmids of different sizes--effects of pH, phosphate, and dissolved oxygen. *Biotechnol. Bioeng.* 34(3):309–19

117. Scanlan PD, Baquero F, Levin BR. 2024. Short-sighted evolution of virulence for invasive gut microbes: From hypothesis to tests. *Proc. Natl. Acad. Sci. U. S. A.* 121(49):e2409905121

118. Schachner-Groehs I, Koller M, Leopold M, Kolm C, Linke RB, et al. 2024. Linking antibiotic resistance gene patterns with advanced faecal pollution assessment and environmental key parameters along 2300 km of the Danube River. *Water Res.* 252:121244

119. Schwab F, Gastmeier P, Meyer E. 2014. The warmer the weather, the more gram-negative bacteria - impact of temperature on clinical isolates in intensive care units. *PloS One*. 9(3):e91105

120. Serrana JM, Nascimento FJA, Dessirier B, Broman E, Posselt M. 2025. Environmental drivers of the resistome across the Baltic Sea. *Microbiome*. 13(1):92

121. Sherburne CK, Lawley TD, Gilmour MW, Blattner FR, Burland V, et al. 2000. The complete DNA sequence and analysis of R27, a large IncHI plasmid from Salmonella typhi that is temperature sensitive for transfer. *Nucleic Acids Res.* 28(10):2177–86

122. Sieburth JM. 1960. Acrylic acid, an “antibiotic” principle in Phaeocystis blooms in antarctic waters. *Science*. 132(3428):676–77

123. Singer AC, Shaw H, Rhodes V, Hart A. 2016. Review of Antimicrobial Resistance in the

983 Environment and Its Relevance to Environmental Regulators. *Front. Microbiol.* 7:
984 124. Sobecky PA, Mincer TJ, Chang MC, Helinski DR. 1997. Plasmids isolated from marine
985 sediment microbial communities contain replication and incompatibility regions unrelated to
986 those of known plasmid groups. *Appl. Environ. Microbiol.* 63(3):888–95
987 125. Stalder T, Top E. 2016. Plasmid transfer in biofilms: a perspective on limitations and
988 opportunities. *Npj Biofilms Microbiomes*. 2(1):1–5
989 126. Thulin E, Thulin M, Andersson DI. 2017. Reversion of High-level Mecillinam Resistance
990 to Susceptibility in Escherichia coli During Growth in Urine. *EBioMedicine*. 23:111–18
991 127. Udekwu KI, Parrish N, Ankomah P, Baquero F, Levin BR. 2009. Functional relationship
992 between bacterial cell density and the efficacy of antibiotics. *J. Antimicrob. Chemother.*
993 63(4):745–57
994 128. Uhlin BE, Molin S, Gustafsson P, Nordström K. 1979. Plasmids with temperature-
995 dependent copy number for amplification of cloned genes and their products. *Gene*. 6(2):91–
996 106
997 129. Vermeulen N, Keeler WJ, Nandakumar K, Leung KT. 2008. The bactericidal effect of
998 ultraviolet and visible light on Escherichia coli. *Biotechnol. Bioeng.* 99(3):550–56
999 130. Vincent J, Tenore A, Mattei MR, Frunzo L. 2024. Modelling Plasmid-Mediated
1000 Horizontal Gene Transfer in Biofilms. *Bull. Math. Biol.* 86(6):63
1001 131. Walter GM, McGuigan K. 2023. Predicting the future. *eLife*. 12:e91450
1002 132. Wang M, Tian Y, Xu L, Zhang F, Lu H, et al. 2022. High Osmotic Stress Increases
1003 OmpK36 Expression through the Regulation of KbvR to Decrease the Antimicrobial
1004 Resistance of Klebsiella pneumoniae. *Microbiol. Spectr.* 10(3):e0050722
1005 133. Wang Q, Wei S, Silva AF, Madsen JS. 2023. Cooperative antibiotic resistance facilitates

horizontal gene transfer. *ISME J.* 17(6):846–54

134. Webber MA, Whitehead RN, Mount M, Loman NJ, Pallen MJ, Piddock LJV. 2015. Parallel evolutionary pathways to antibiotic resistance selected by biocide exposure. *J. Antimicrob. Chemother.* 70(8):2241–48

135. Wilkinson JL, Boxall ABA, Kolpin DW, Leung KMY, Lai RWS, et al. 2022. Pharmaceutical pollution of the world's rivers. *Proc. Natl. Acad. Sci. U. S. A.* 119(8):e2113947119

136. Williams CE, Williams CL, Logan ML. 2023. Climate change is not just global warming: Multidimensional impacts on animal gut microbiota. *Microb. Biotechnol.* 16(9):1736–44

137. 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(1):1599

138. Wong F, Wilson S, Helbig R, Hegde S, Aftenieva O, et al. 2021. Understanding Beta-Lactam-Induced Lysis at the Single-Cell Level. *Front. Microbiol.* 12:712007

139. Wood JM. 2011. Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. *Annu. Rev. Microbiol.* 65:215–38

140. Xiang Y, Jia M, Xu R, Xu J, He L, et al. 2024. Carbamazepine facilitated horizontal transfer of antibiotic resistance genes by enhancing microbial communication and aggregation. *Bioresour. Technol.* 391(Pt A):129983

141. Zhang D, Li J, Zhang G, Xu Y. 2025. Decoding the trajectory of antibiotic resistance genes in saline and alkaline soils: Insights from different fertilization regimes. *Environ. Int.* 195:109247

142. Zhang Y, Cai Y, Zhang B, Zhang Y-HPJ. 2024. Spatially structured exchange of

metabolites enhances bacterial survival and resilience in biofilms. *Nat. Commun.* 15(1):7575

143. Zhang Y, Cheng J, Lin C, Li F, Zhang X, et al. 2024. Spatial heterogeneity determines the gastrointestinal microbiome signatures and ecological processes that govern bacterial community assembly in sheep. *Microbiol. Spectr.* 13(2):e01110-24

144. Zhang Y, Xue J, Luo Y, Muhammad I, Dong H, et al. 2025. Acidifiers promoted antibiotic resistance gene transfer via plasmid conjugation. *J. Environ. Chem. Eng.* 13(3):117203

145. Zhao W, Ye C, Li J, Yu X. 2024. Increased risk of antibiotic resistance in surface water due to global warming. *Environ. Res.* 263(Pt 2):120149

146. Zhao Z, Baltar F, Herndl GJ. 2024. Decoupling between the genetic potential and the metabolic regulation and expression in microbial organic matter cleavage across microbiomes. *Microbiol. Spectr.* 12(5):e0303623

147. Zhu S, Yang B, Wang Z, Liu Y. 2023. Augmented dissemination of antibiotic resistance elicited by non-antibiotic factors. *Ecotoxicol. Environ. Saf.* 262:115124

148. Zhu S, Yang B, Yu F, Zhang J, Wang Z, Liu Y. 2024. Investigation of the impact of widely used pesticides on conjugative transfer of multidrug resistance plasmids. *J. Hazard. Mater.* 478:135436

149. Ziegelhoffer EC, Donohue TJ. 2009. Bacterial responses to photo-oxidative stress. *Nat. Rev. Microbiol.* 7(12):856–63

150. Zinser ER, Kolter R. 2004. Escherichia coli evolution during stationary phase. *Res. Microbiol.* 155(5):328–36

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1054