PERSPECTIVE

Antibiotics and Antibiotic Resistance Genes in Natural Environments

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The large majority of antibiotics currently used for treating infections and the antibiotic resistance genes acquired by human pathogens each have an environmental origin. Recent work indicates that the function of these elements in their environmental reservoirs may be very distinct from the "weapon-shield" role they play in clinical settings. Changes in natural ecosystems, including the release of large amounts of antimicrobials, might alter the population dynamics of microorganisms, including selection of resistance, with consequences for human health that are difficult to predict.

The success of antibiotics for treating infections and, conversely, the risk that antibiotic resistance poses for human health has meant that research in this area has focused primarily on their role within clinical settings. In contrast, the function of antibiotics in natural (nonclinical) environments has received relatively little attention. Most antibiotics used for treating infections are produced by environmental microorganisms, meaning that genes for antibiotic resistance must also have emerged in nonclinical habitats (*1*). A better understanding of the ecological role for antibiotics and antibiotic resistance in nonclinical environments (Fig. 1) may eventually help to predict and counteract the emergence and future evolution of resistance (*2*).

Ecological Role for Antibiotics in Natural Environments

The successful finding of antibiotic-producing microorganisms in soil led to the idea that a primary ecological role for antibiotics is likely to be in inhibiting the growth of competitors (*3*). Because any chemical entity can be toxic at a sufficiently high concentration, it seems plausible that molecules selected by the pharmaceutical companies for their antibiotic properties at therapeutic concentrations would also have distinct functions at the lower concentrations probably encountered in nature (Fig. 2). For example, low concentrations of antibiotics trigger specific transcriptional changes that are independent of the bacterial stress response pathways (*4*) and may have beneficial consequences for the bacteria that modulate the interactions within microbial communities (*5*, *6*). Similarly, molecules formerly classified as delivering signals for intermicrobial communication have subsequently been found to possess demonstrable antibiotic activity (*5*). This capacity for signaling at low concentrations has further clinical implications because subinhibitory concentrations of antibiotics could favor bacterial virulence under certain conditions (*6*, *7*).

Why So Many Antibiotic Resistance Genes?

The huge number of antibiotic resistance genes found in the environment (*8*) raises the obvious question of why so many have evolved. Recent work has shown a pronounced breadth of utilization of antibiotics as a source of nutrients by bacteria, and it seems natural that this should have led to considerable levels of resistance (*9*). Equally, it seems clear why antibiotic-producing microorganisms should possess determinants to help them resist the action of the antibiotics they produce, but it is less obvious why bacteria that

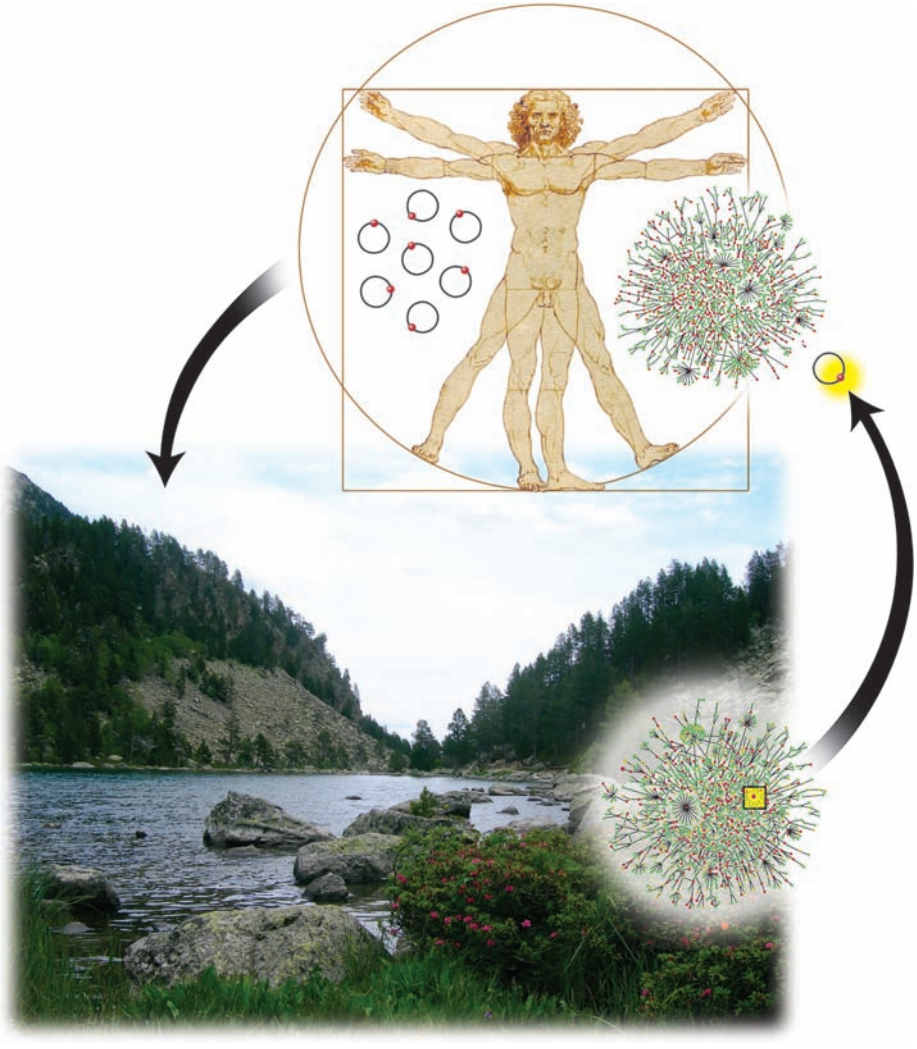

Fig. 1. Transfer of antibiotic resistance genes from natural clinical environments. Most antibiotic resistance genes acquired through horizontal gene transfer have been originated in environmental microorganisms. In their original host, these determinants (red dot) form part of integrated regulatory and metabolic networks. After their dissemination (circle), their integration in the novel metabolic networks would be difficult, and their only role will be resistance. The reintroduction of plasmid-encoded antibiotic resistance determinants in natural environments, together with the changes they suffer as the consequence of human activities, might be relevant for the future evolution and dissemination of antibiotic resistance determinants in bacterial pathogens.

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do not themselves produce antibiotics should also possess multiple resistance determinants (*10*). For example, multidrug resistance (MDR) efflux pumps are present in all organisms and can exist in large numbers within a single microorganism (*11*). Because their antibiotic resistance profiles overlap, it seems unlikely that all are required to resist antibiotics, and we now know that MDR elements are involved in other processes such as detoxification of metabolic intermediates, virulence, and signal trafficking, among other functions (*12*–*14*). What's more, a low-specificity enzyme might be classified as part of the antibiotic resistance machinery, even if it had evolved in the degradation or modification of metabolites (*15*). Thus, the dual nature of antibiotics as both signals and weapons can explain how genes, which may not have evolved their primary function in resistance, can nevertheless contribute to the protection from antibiotic threat.

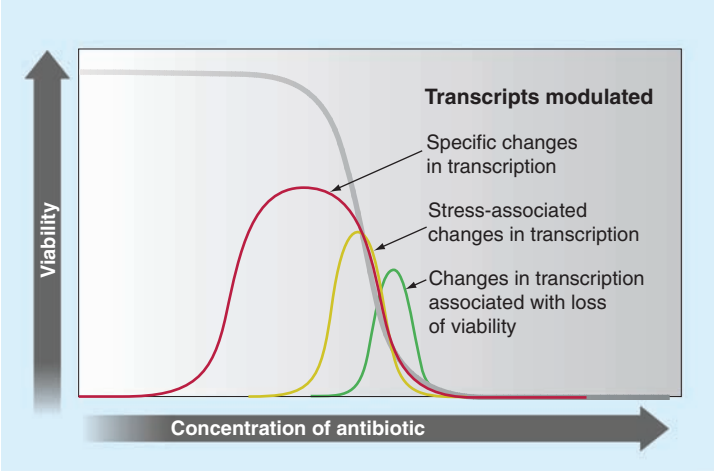

Fig. 2. Effect of antibiotics on bacterial gene expression. Antibiotics trigger concentration-dependent changes in bacterial transcription. At low concentrations, the expression of several genes changes, and this has been interpreted as a signaling effect. At higher concentrations, stress responses sum up to the changes in transcription and, at the highest concentrations, the changes in transcription probably reflect the loss of viability [modified from (*5*)].

Environmental Intrinsic Resistant Bacteria

Development of antibiotic resistance as the consequence of mutation (*16*) or horizontal gene transfer (*17*) is considered to have been driven by the relatively recent selective pressure of antibiotics used in therapeutic settings. Nevertheless, some bacterial species possess an intrinsically low susceptibility to antibiotics. Such bacteria have environmental origins in habitats that do not harbor a high antibiotic load, and those responsible for infections in hospitals (for instance, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, or *Stenotrophomonas maltophilia*) are not themselves antibiotic producers. Free-living opportunistic pathogens often have large genomes that allow the colonization of diverse environments through metabolic versatility that helps to degrade and resist toxicity of compounds present in these ecosystems. This can include large numbers of biodegradative enzymes that cooperate in the modification and utilization of antibiotics as a food resource (*9*). Additionally, efflux pumps—originally involved in signal trafficking or resistance to toxic compounds produced by plants or rhizosphere-associated microbiota—can be used for effluxing antibiotics as well.

From Natural to Clinical Environments: A Functional Shift

In clinical environments, pathogenic and commensal bacteria are challenged with high concentrations of antibiotics. Thus, the function of the antibiotics in such ecosystems has been uniquely imposed by humans and is aimed solely at inhibiting bacterial proliferation. As a result, antibiotic resistance emerges to specifically overcome this inhibition. Drawing once again on the example of a protein able to function in modifying an antibiotic beyond its original function, it seems likely that this could serve as an antibiotic resistance determinant and evolve over shorter time frames in the clinic. For instance, *Providencia struartti* possesses an enzyme (2′-*N*-acetyltransferase) that modifies bacterial peptidoglycan, and the similarity of its substrate to gentamycin enables the enzyme to modify both its regular substrate and the antibiotic (*15*). Acquisition of the gene by another bacteria via plasmid transfer would place the encoded enzyme outside its normal biochemical context, and its unique function would then solely be acquired resistance to gentamycin (*18*).

The example described above addresses two characteristics that could allow functional shifting of antibiotic resistance determinants. Both refer to such elements as being "out of context." First, regulation of their expression in the pathogen is not equivalent to that in the original host. A key element in the establishment of metabolic networks is the fine-tuned regulation of the elements involved in response to external signals. Such regulation is often lost in the case of horizontally transferred antibiotic resistance genes, which are frequently expressed constitutively. For example, gene-capture units called integrons are involved in MDR and contain arrangements of antibiotic resistance genes, the expression of which is driven by a strong promoter (*19*). Second, the biochemical activity of a given protein encoded by a transferred gene is not inserted in the metabolic network where it evolved (Fig. 1).

Environmental Changes and the Evolution of Antibiotic Resistance

Natural environments represent reservoirs of antibiotic resistance genes, such that changes in these ecosystems might be relevant for the emergence of previously unknown resistance determinants in bacterial pathogens. It is surprising, therefore, that although there is a substantial concern over the potential effect of antibiotic resistance genes used for modifying organisms that can be released in the environment (*20*), the effect that changes to the environment may have on the population dynamics of bacteria and their antibiotic resistance genes has received much less attention (*1*). In this respect, it is worth noting that the antibiotic resistance genes most frequently used for genetic engineering are already widely disseminated among bacterial pathogens. In contrast, previously unrecognized antibiotic resistance genes that may emerge in the future already exist in many as yet ignored environmental organisms (*2*).

Whether anthropogenic changes of the environment might enrich the population of resistant bacteria and facilitate the transfer of resistance genes to human pathogens will be important to address in the future. The clearest example of this potential effect is contamination by antibiotics themselves. Antibiotics are currently used widely, not just for the treatment of human infections, but also in agriculture and animal/fish farming (*21*), with the possibility that high amounts of such compounds may find their way into natural habitats (*22*). An example of the effect of antibiotic contamination is that of the quinolone resistance gene *qnr*, which is present in the chromosomes of waterborne bacteria, where it has a so-far unknown function (*23*). After being integrated in plasmids, where it is constitutively expressed, *qnr* contributes to low-level resistance of its new bacterial host to quinolones (*24*). Recent work has shown that contamination of river waters by quinolones enriches for plasmid-encoded *qnr* genes present in waterborne bacteria, in such a way that may allow a first step in the transfer of this gene to human pathogens (*25*). Other types of contamination may also select for antibiotic resistance in nature. For instance, heavy metal pollution can select for antibiotic resistance (*26*), and stress conditions, as found in polluted environments, have the potential to increase recombination and horizontal gene transfer in a way that favors the dissemination of antibiotic resistance genes (*27*).

The presence of human commensal (and human-pathogenic) bacteria in the environment can be considered yet another form of contamination. Because any antibiotic resistance gene needs to coexist in the same environment as the human pathogen to which it may transfer, the increase in human population and the widespread lack of efficient wastewater treatment bring with them a risk of transfer of antibiotic resistance. Finally, it seems reasonable to speculate that a human-driven increase in the concentrations of antibiotics in natural ecosystems may not only influence antibiotic resistance, but also affect the broader microbial population dynamics in different natural environments.

Natural (nonclinical) habitats represent the main source of antibiotics and where antibiotic resistance has primarily evolved. The functional role these elements play in such environments is likely to be distinct from their "weapon/shield" function in clinical settings. In spite of the ecological relevance that antibiotics and resistance determinants have in nonclinical environments, there remains much to learn about the effect that human-driven changes of natural ecosystems may have on the evolution and dissemination of resistance in nature. Yet, the relevance this is likely to have for the future of human health is clear.

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PERSPECTIVE

Outwitting Multidrug Resistance to Antifungals

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The economic cost of fungal infection and its mortality associated with multidrug resistance remain unacceptably high. Recent understanding of the transcriptional regulation of plasma membrane efflux pumps of modest specificity provides new avenues for the development of broad-spectrum fungicides. Together with improved diagnosis and indirect intervention via inhibition of the energy supply for drug efflux, we envisage multifunctional azole analogs that inhibit not only ergosterol biosynthesis and drug efflux-pump activity but also activation of the transcriptional machinery that induces drug efflux-pump expression.

Eight hundred million years of evolution have generated ~1.5 million fungal species that occupy many distinct ecological niches, yet only ~300 fungi cause disease in humans (*1*). The identification of antifungals that act specifically against these pathogens is a particular challenge because of fungal diversity, individualized pathways for infection, and fungal use of multiple mechanisms that circumvent exogenous toxins. These highly regulated mechanisms include innate resistance to specific antifungal drugs, formation of biofilms, selection of spontaneous mutations that increase expression or decrease susceptibility of the drug target (*2*), stress-related tolerance that enhances short-term survival (*3*, *4*), modification of chromosomal ploidy (*5*), and overexpression of multidrug efflux pumps (*6*). Fortunately, compared with infections caused by drug-resistant bacteria, those caused by resistant fungal pathogens and their spread to other patients occur relatively infrequently. However, the economic cost of fungal infection and its associated mortality, especially in debilitated and high-investment patients, remain unacceptably high.

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A Clinical Perspective

The most prominent fungal pathogens affecting humans include *Aspergillus fumigatus*, *Candida albicans*, *C. glabrata*, *C. parasilosis*, *C. tropicalis*, *C. krusei*, and *Cryptococcus neoformans* (*7*). Although the skin, mucosal surfaces, and immune system usually provide robust defenses, weakened immunodefenses dramatically increase susceptibility to debilitating and life-threatening opportunistic fungal infections. Fungal infections are normally treated with a modest repertoire of drugs derived from five antifungal classes that target DNA and RNA synthesis, ergosterol, the ergosterol biosynthetic pathway, or the biosynthesis of the cell-wall component 1,3-β-D-glucan (Table 1). Unfortunately, the prophylactic use of fungistatic azoles such as fluconazole has been associated with an increased frequency of innate or acquired drug resistance in clinical isolates and the selection of non-*albicans Candida*, non-*fumigatus Aspergillus*, opportunistic yeastlike fungi, zygomycetes, and hyaline molds. Despite the fact that broader-spectrum third-generation azole drugs and the more expensive echinocandin class of antifungals prevent an increased proportion of life-threatening infections, *Candida* species remain the fourth most common cause of hospital-acquired bloodstream infection and kill 40% of those patients, whereas disseminated *Aspergillus* infections kill up to 80% of affected patients.

Mechanisms of Multidrug Resistance

Because of its economic and clinical impact, a focus on multidrug resistance rather than resistance to specific antifungals in pathogenic fungi is timely. Multidrug resistance, called pleiotropic drug resistance (PDR) in *Saccharomyces cerevisiae*, is an ancient phenomenon that preceded the modern use of antifungals (*8*). The adenosine triphosphate (ATP)–binding cassette (ABC) and major facilitator superfamily (MFS) transporter families responsible for multidrug resistance operate in all fungi. We distinguish among the transporters that belong to different species by using the prefix Sc for *S. cerevisiae*, Cg for *C. glabrata*, or Ca for *C. albicans*.

Saccharomyces cerevisiae. PDR in *S. cerevisiae* is the best-understood multidrug resistance mechanism in fungi. Point mutations conferring resistance to chemically diverse drugs (including azoles) have been mapped in genes encoding the zinc-

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