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Toward a Comprehensive Strategy to Mitigate Dissemination of Environmental Sources of Antibiotic Resistance

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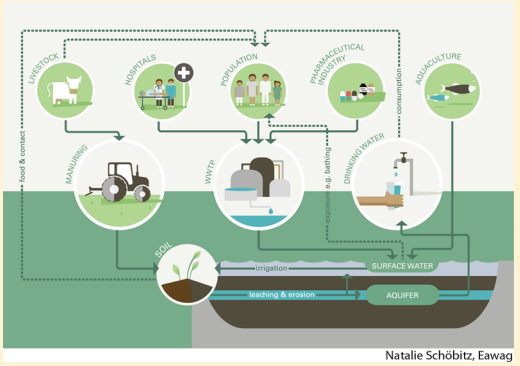
ABSTRACT: Antibiotic resistance is a pervasive global health threat. To combat the spread of resistance, it is necessary to consider all possible sources and understand the pathways and mechanisms by which resistance disseminates. Best management practices are urgently needed to provide barriers to the spread of resistance and maximize the lifespan of antibiotics as a precious resource. Herein we advise upon the need for coordinated national and international strategies, highlighting three essential components: (1) Monitoring, (2) Risk Assessment, and (3) Mitigation of antibiotic resistance. Central to all three components is *What* exactly to monitor, assess, and mitigate? We address this question within an environmental framework, drawing from fundamental microbial ecological processes driving the spread of resistance.



Antibiotic resistance is one of the greatest public health challenges facing humanity in the 21st Century. Current assessments suggest that antibiotic resistant bacteria (ARB) are responsible for at least 23 000 deaths per year in the U.S., ~25 000 deaths per year in Europe, and hundreds of thousands of deaths in lesser-developed countries and regions.[1,2] The European Commission estimates that the costs associated with antibiotic resistant infections exceed €1.5 billion per year,[3] while in the U.S. one estimate suggests the costs are a staggering $55 billion per year.[4] Unfortunately, these startling numbers are only getting worse with the continued emergence and dissemination of multidrug resistant (MDR) "superbugs" that simultaneously exhibit resistance to multiple antibiotic classes. (Note: We define MDR and many other technical terms in the Glossary.) For example, a MDR strain of *Escherichia coli* resistant to colistin (polymyxin E), an antibiotic of last resort, was recently found in the U.S.[5] after being initially detected only a few years before in China.[6] These trends collectively stoke the fear that our globalized society could essentially enter a postantibiotic era similar to that before antibiotics were available and deaths due to bacterial infections were commonplace.[7]

The antibiotic resistance problem has not gone unnoticed and the level of national and international cooperation building toward addressing this challenge is encouraging. Internationally, the World Health Organization (WHO) has developed a Global Action Plan on Antimicrobial Resistance[8] and many other regional and national plans are being developed and implemented.[3,9] Core to these strategies is the prudent stewardship of the use of antibiotics[10] along with improved education of medical professionals, the agricultural sector, veterinarians, and the public regarding the importance of

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avoiding misuse and overuse of antibiotics. Antibiotics are the quintessential double-edged sword: on one hand they are an essential life-saving weapon, on the other hand, misuse and overuse stimulate bacterial evolution and the spread of resistance.[11] Slowly and inexorably, the more we use antibiotics, the greater the chance they will lose their effectiveness over time.

The ideal strategy to combat antibiotic resistance is to reduce the rate at which antibiotic resistant bacteria evolve and spread.[12] This is a challenging task, but a necessary one that is beginning to gain traction in various national and international action plans. Many of these plans specifically advocate for a "One Health" perspective that simultaneously addresses the impacts of antibiotic resistance on humans, animals, and the environment. This construct is useful because it incorporates a systems perspective that considers not only the epidemiology of resistance, but also the underlying socioeconomic, political, biological, and ecological factors that influence its spread.[13] Herein we explicitly focus on the environmental dissemination of antibiotic resistance. As is true of many other environmental grand challenges (e.g., climate change, equitable water supply) antibiotic resistance is a highly complex problem for which resilient solutions require buy-in from a range of different actors. Environmental scientists and engineers must learn from past successes and failures in dealing with multifaceted problems and bring that knowledge to bear on antibiotic resistance. For instance, we have learned a lot about how to mitigate pollution arising from distributed sources and some limited successes have been achieved through improved manure and fertilizer control, wetland management, and nutrient retention and recovery in regions such as the Mississippi river basin[14] and the Chesapeake Bay.[15] These and similar knowledge provide a foundation upon which appropriate control and mitigation strategies can be developed. In contrast to other pollutants, however, antibiotic resistance is autoreplicative.[16] This means that antibiotic resistance genes (ARGs) that enable normally sensitive bacteria to survive in the presence of an antibiotic are readily shared between bacterial cells and that both the resistant bacteria and the ARGs that they carry can rapidly multiply. This characteristic makes control of resistance dissemination a daunting challenge.

Globally agreed upon management practices to limit the spread of antibiotic resistance via environmental pathways present an important opportunity. Ideally, such measures have the potential to impart other benefits, such as improved water purification, soil conditioning, and nutrient management.[17] Herein we expand upon three essential components needed to advance strategies for combatting the spread of antibiotic resistance: (1) Monitoring, (2) Risk Assessment, and (3) Mitigation. Central to all three components is *What* exactly to monitor, assess, and mitigate? To answer this question, we must explicitly consider the root sources and causes of antibiotic resistance and, in particular, the role of environmental processes.

ORIGIN AND EVOLUTION OF ANTIBIOTIC RESISTANCE

Antibiotic resistance has likely been around nearly as long as bacteria themselves, or approximately three billion years.[18] While we as humans think of antibiotics as life-saving drugs, in reality the vast majority of these drugs are derived from natural compounds produced by microbes often for their own "selfish" purpose of fending off other bacteria and promoting themselves, or for uses entirely unrelated to bacterial inhibition.[16] Highly specialized resistance genes have evolved in response to the presence of these antibiotics. Accordingly, it is not surprising that pristine Arctic soil cores, frozen long before humans began mass producing and using antibiotics, are host to a wide variety of ARGs.[19,20] The widespread distribution of ARGs contributes an underlying baseline ARG level, or resistome, to all natural and human-impacted habitats.[21,22] However, following the advent of the antibiotic age in the 1940s it has become clear that anthropogenic inputs of antibiotics distinctly alter affected environments. For instance, in the South Platte River Basin in Colorado it was found that *sul*1 ARGs (see Glossary for definitions of this and other ARGs) displayed a near perfect correlation with wastes from upstream wastewater treatment plants and livestock operations.[23] Similar anthropogenic impacts have been noted for several *tet* ARGs in the Almendares River in Cuba,[24] class 1 integrons in the River Thames in the UK[25] and the *sul*1 gene in Swiss lakes.[26] Further, examination of archived Dutch soils spanning the pre- to postantibiotic era noted up to a 15-fold increase in the levels of 18 ARGs encoding for resistance to extended spectrum beta-lactamases, tetracylines, and erythromycins.[27] A recent comprehensive survey of ARGs across a range of environments demonstrated that ARG levels are elevated in areas with intensive antibiotic use relative to natural background levels.[28] Thus, while antibiotic resistance itself is a natural phenomenon, human activities often correlate with elevated levels of ARGs across a variety of environments.

MONITORING ANTIBIOTIC RESISTANCE AS AN ENVIRONMENTAL CONTAMINANT

Given that the spread of antibiotic resistance is undesirable and that there are demonstrated human impacts on the resistome, there is growing interest in and attention to monitoring resistance as an environmental "contaminant".[29,30] Ecological niches that are nutrient rich and characterized by high bacterial concentrations are ideal settings for resistance to develop and spread.[31] Logical environmental monitoring points with such characteristics include wastewaters from homes, hospitals, and antibiotic manufacturing facilities as well as livestock and biosolids (Figure 1). However, the key question remains - *What* to monitor?

Antibiotic resistance monitoring efforts have been developed in the food and agricultural sectors and have the potential to be adapted for environmental monitoring. For example, the U.S. Food and Drug Administration, the U.S. Centers for Disease Control and Prevention, and the U.S. Department of Agriculture have been working together to monitor antibiotic resistance by tracking changes in the antibiotic susceptibility of a group of enteric (intestinal) bacteria in U.S. meat products, people, and farm animals through the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) program.[32] In Europe, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) has closely mapped resistance rates across Denmark through profiling of antibiotic consumption as well as quantification of resistant bacteria from animals, food, and humans.[33] A similar program exists in Switzerland, where the ANRESIS database tracks both antibiotic consumption and resistance in sentinel bacteria in human and veterinary contexts.[34] Such programs generally rely on bacterial culturing with a focus on intestinal pathogens (human disease causing bacteria) and bacterial indicators of interest. There are many advantages to culture-

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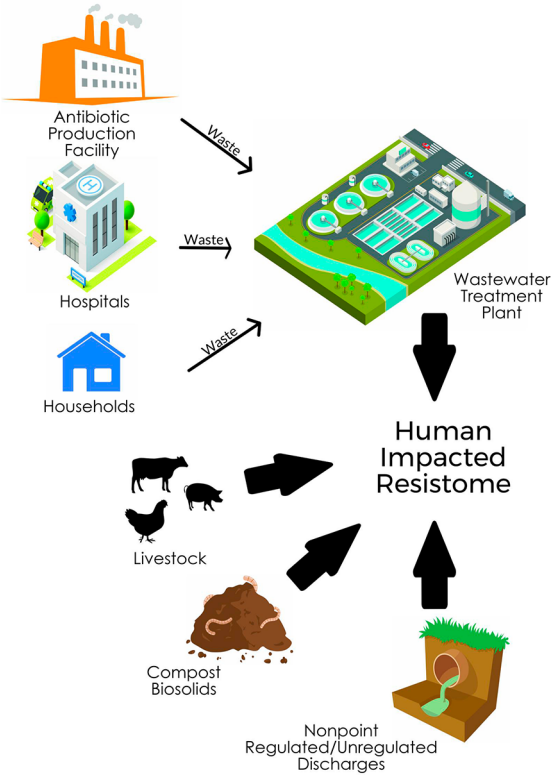

Figure 1. Key environmental matrices and flows that should be monitored to quantify environmental antibiotic resistance dissemination. Small arrows reflect waste flows from environmental reservoirs to a wastewater treatment plant. Large arrows reflect direct impacts of different reservoirs on the human-impacted resistome.

based monitoring such as the confirmation that living bacteria are present, the ability to measure living pathogens, and in combination with molecular microbiology, the ability to link specific ARGs to their bacterial hosts.[35] One of the strategic objectives of the WHO Global Action Plan for Antimicrobial Resistance is to strengthen knowledge through surveillance and research.[8] An expert WHO workshop was convened with a key recommendation that *E. coli* is a good indicator of resistance. *E. coli* is widely studied, it is representative of many pathogens, and methods for its isolation are accessible for use in lesser-developed countries.[36] However, one challenge in adapting culture-based methods for environmental monitoring is that nonpathogenic, environmental bacteria may represent important reservoirs of resistance, but the vast majority of these are not easily cultured and thus may avoid detection.[37] These environmental bacteria have the potential to share their ARGs with pathogens or the normal commensal bacteria that coexist within and on humans.

Circumventing culturing issues, substantial strides have been made in the development of molecular tools that are well-suited for tracking antibiotic resistance.[38] Individual ARGs are now routinely tracked in environmental systems using quantitative polymerase chain reaction (qPCR). This is an attractive approach because the quantitative information collected enables one to examine potential correlations between ARG concentrations and chemicals (e.g., metals, antibiotics) or other agents that may select for resistant bacteria and then evaluate mitigation strategies. However, qPCR is limited in that it is generally only able to examine a few ARGs at a time.[23] Given that there are thousands of known ARGs,[39] this then begs the question *Which* ARGs or mobile genetic elements to target? Many early studies focused on detection of sulfonamide and tetracycline ARGs,[40] while more recent efforts have concentrated on ARG classes of emerging health and clinical concern such as carbapenemases (e.g., $bla_{\text{NDM-1}}$,[41] $bla_{\text{KPC-2}}$[42]) and fluoroquinolone targeting ARGs (e.g., *qnr*S[43]). Often the usefulness of an ARG as a trackable indicator (e.g., the abundant *sul*1 or *tet*W genes) may be at odds with their potential risk, as many genes of clinical concern are not (yet) abundant in environmental samples and thus present a current challenge for monitoring purposes. qPCR arrays, versions of which have been applied to simultaneously track up to 285 ARGs in environmental samples (e.g., animal manure,[44] wastewater irrigated soils,[45] and coastal waters[46]), are one means of circumventing this issue. Currently the StARE (Stopping Antibiotic Resistance Evolution) monitoring program is utilizing qPCR array to characterize the resistomes of wastewater treatment plants across Europe.[47] However, qPCR arrays have a higher detection limit, are more difficult to validate, and are more costly than traditional qPCR.

While qPCR has provided a glimpse of what is possible through molecular monitoring of antibiotic resistance, the emergence of high-throughput DNA sequencing technologies suggests a path to the future. Shotgun metagenomic sequencing offers the capacity to profile the full complement of DNA in a sample without the a priori selection of target ARGs.[48] In particular, pyrosequencing and Illumina HiSeq technology have been applied to identify and monitor thousands of ARGs in wastewater[49,50] and heavily polluted river sediments.[51] However, metagenomics provides an estimate of relative abundance, not absolute abundance, and detection limits are typically orders of magnitude higher than qPCR. These issues, when coupled with more time-consuming and more costly analyses (which are continually declining), provide significant barriers to the application of the metagenomic approach for monitoring purposes. As the technologies evolve and these issues are resolved, it will be critical to agree upon standard means of interpreting and reporting metagenomic data. This task begins with defining the specific parameters (e.g., alignment lengths, comparable amino acid identities) used to identify an ARG as an ARG.[49] The Comprehensive Antibiotic Resistance Database (CARD)[39] is widely considered the most up-to-date resistance gene database, but it is not specifically curated with the purpose of environmental monitoring and thus a number of research groups have developed in-house methods to eliminate database redundancies. The recently developed ARGs-OAP pipeline is one such tool that provides an online platform for detecting ARGs in metagenomic data sets.[52] This pipeline incorporates a nonredundant database that couples CARD with the older Antibiotic Resistance Database (ARDB)[53] to better facilitate ARG identification. These in-house approaches must be standardized to make cross-lab comparisons possible.

Even after ARGs have been identified, there is still the issue of how to best analyze and interpret the data. For example, consideration is needed regarding which assessment is most relevant: Total ARG abundance? Relative abundance of total ARGs (i.e., normalized to bacterial 16S rRNA gene, a proxy for bacterial cell number, or cell number)? Diversity of ARGs? Or specific ARGs of interest? For example, ARGs encoding resistance to last resort antibiotics (e.g, vancomycin, extended spectrum beta lactams, carbapenems, or colistin) are arguably of greater concern than ARGs encoding resistance to

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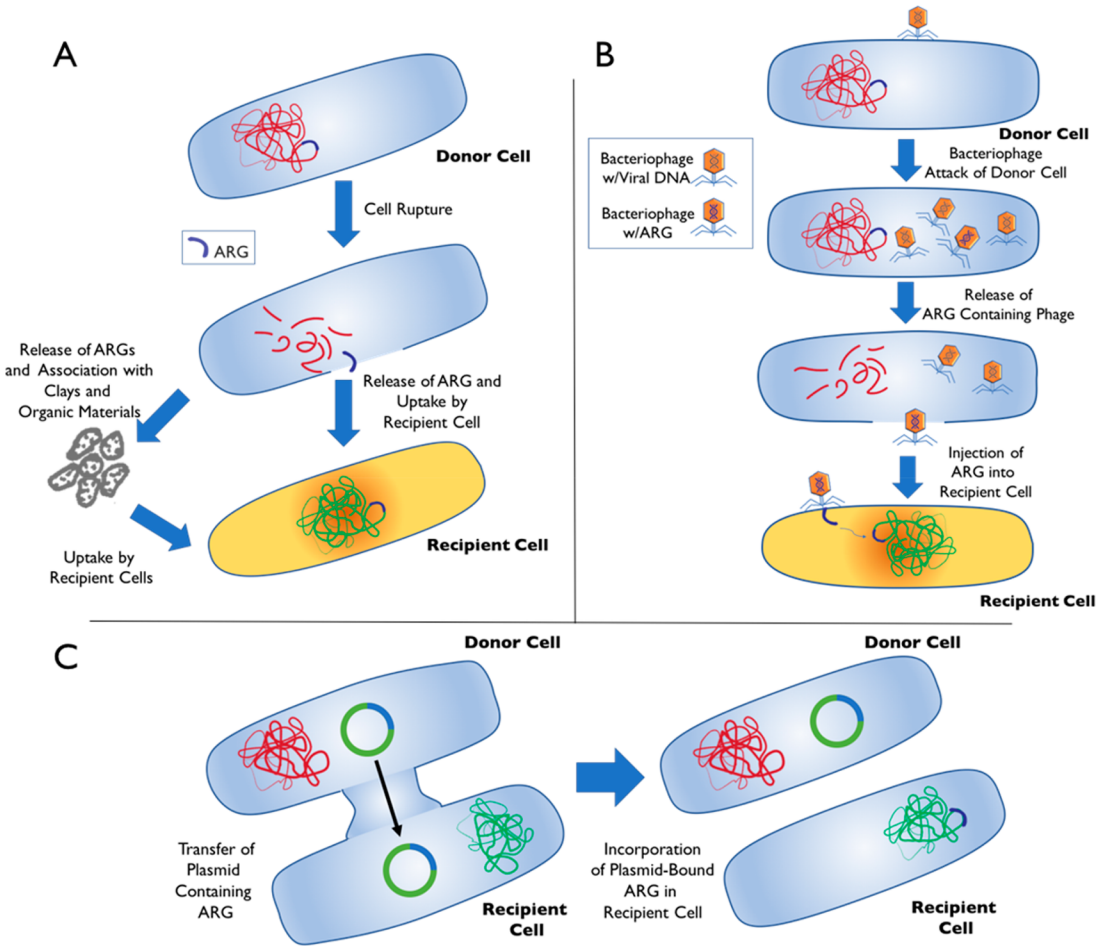

Figure 2. Mechanisms of horizontal gene transfer (HGT). (A) Transformation, (B) Transduction, and (C) Conjugation. Transformation is possible when cells are injured and release DNA. This extracellular DNA can be taken up by a competent recipient cell. In some cases, extracellular DNA associates with and is protected by clay or environmental macromolecules prior to uptake. Transduction involves a bacteriophage intermediary that mistakenly acquires bacterial DNA following cell lysis. If this DNA encodes for antibacterial resistance and is injected into a recipient cell it can combine with recipient cell DNA. Conjugation requires bacterium-bacterium conjugation. During conjugation, a plasmid (shown) or another mobile genetic element that encodes for resistance is transferred between cells.

tetracycline, which has largely lost clinical application.[54] On the other hand, a strong case can be made that mobile genetic elements (MGEs) associated with horizontal gene transfer (HGT), including plasmids, integrons, and prophages, are really the greatest concern.[21] The underlying logic of this argument is that it is the transfer of ARGs to new hosts and the corresponding emergence of new resistant strains that is more problematic than the documentation of existing resistant strains. A recent critical review made the case that the class 1 integron *int*I1 is a particularly suitable target for this purpose as it correlates well with anthropogenic inputs and other "pollution" markers.[55] This hypothesis was recently supported by studies documenting a strong correlation between *int*I1 levels and ARGs in coastal estuaries[46] as well as in 64 environmental samples acquired across eight different ecosystems.[56]

It is essential to be aware of and take into account potential limitations when developing and applying any ARG monitoring scheme. For example, standard DNA extraction techniques may capture both intracellular and extracellular DNA and these will not be differentiated downstream. Recent work suggests that extracellular DNA is fairly abundant in certain environments, such as sludges and sediments.[57,58] Unfortunately, methods to differentiate intracellular and extracellular DNA are not standardized and are difficult to validate even though extracellular DNA remains relevant to risk because it can be taken up by bacteria via transformation (Figure 2).[59] To verify the functionality of extracellular DNA, transformation assays can be performed on the sample of interest, as was done by Luo and colleagues to verify that bla_{NDM-1} escaping wastewater disinfection in China outlasted the discharged bacteria harboring it and was still capable of being taken up by and expressed within recipient hosts.[41,60] Wigginton and colleagues demonstrated that while transformation assays provide the gold standard to assess the functionality of environmental DNA, qPCR data can serve as a conservative proxy for ARG transferability.[61]

A significant body of literature documents the occurrence of ARB and ARGs in a multitude of environments. Concerted efforts are now required to critically evaluate the reliability and comparability of this data to identify the ARBs and ARGs that are most relevant to monitoring. The same is true with respect to chemicals (e.g., metals, antibiotics) or other agents that may select for resistant bacteria.[62,63] In Europe, the COST (Cooperation in Science and Technology) Action DARE (Detecting Evolutionary Hotspots of Antibiotic Resistance in

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Europe) group consensus recommends comprehensive monitoring of several potential selectors, including antibiotics and metals, as well as ARGs that represent a cross-section of classes (e.g., *sul*, *bla*, *qnr*, *van*, *erm*, *aac*, *mec*, *aph*, and *tet*), the *int*I1 class 1 integron, as well as *E. coli* and several widely disseminated opportunistic pathogenic bacteria.[64]

ASSESSING RISKS ASSOCIATED WITH ANTIBIOTIC RESISTANCE AS AN ENVIRONMENTAL CONTAMINANT

Ultimately, the ability to accurately and meaningfully assess risks associated with environmental sources and routes of resistance dissemination will be essential to effectively inform policy and target appropriate mitigation strategies. Ideally, measurements of the quantities and types of ARB and ARGs occurring in various media (water, air, soil, food) and measurement of rates of transfer between media can be translated into the likelihood of individuals being exposed and eventually contracting a resistant infection (Figure 3). Much

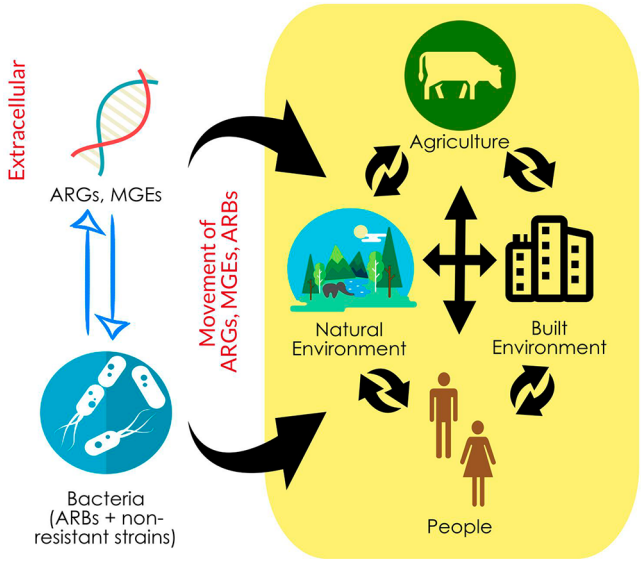

Figure 3. To quantify antibiotic resistance dissemination it is necessary to determine the rates of processes (identified by arrows) dictating antibiotic resistance gene (ARG) and mobile genetic element (MGE) movement and proliferation within the bacterial communities associated with people, agriculture, and the natural and built environments.

progress has been made with this approach with individual pathogenic bacteria, viruses, and protozoa, for example through the framework of quantitative microbial risk assessment (QMRA).[65] However, adapting such a framework to account for the augmented risk associated with the autoreplicative nature of antibiotic resistance presents numerous challenges.

At the heart of the challenge of assessing the risk of environmental sources and pathways of antibiotic resistance is HGT. As noted above, HGT occurs when bacteria share genes with each other. For many ARGs these genes encode for prevention of antibiotic access (e.g., efflux pumps or the impermeabilization of the bacterial membrane), antibiotic target modification, or antibiotic degradation enzymes.[66] As shown in Figure 2, HGT occurs via three primary mechanisms: transformation (uptake of naked environmental DNA), transduction (bacteriophage mediated transfer), and conjugation (bacterial "mating" and sharing of plasmids). Because of these transfer possibilities, ARGs readily transcend their hosts and risk assessment must account for HGT by explicitly focusing on genes and their fates (Figure 3).[37] This basic concept was recently proposed by the "ResCon" risk ranking system in which individual ARGs are assigned a risk value between 1 and 7 depending on a number of considerations.[21] Specifically, ARGs that are more recently evolved, that encode resistance to new antibiotics, or that are associated with MGEs score higher on the risk scale. It has been argued that ARGs that are well-known and have been around for decades are less of a concern than ones that have recently emerged and that potentially pose the next big threat, but are not yet widespread.[67]

A major challenge associated with focusing on emerging ARGs is that they are, inherently, difficult to detect in the environment. Perhaps for such ARGs, quantitative risk modeling is not essential; rather their presence alone raises concern and the impetus for action. However, there is a need for quantitative models to predict which human activities and interventions are most likely to exacerbate or diminish risk, and for such purposes the targeting of more widespread ARGs, MGEs, and hosts may be more logical. For example, consistent with the framework proposed by the DARE COST action, a subset of "indicator" ARGs representative of a range of resistance to antibiotic classes, such as tetracyclines, sulfonamides, macrolides, and beta-lactams, can be used.[64]

Until quantitative ARG risk models are developed, a relative risk approach is one reasonable path forward. Given that ARGs occur at some level even in unperturbed natural environments, due to the previously discussed role of natural antibiotics, it is important to compare systems of interest to their relevant "backgrounds," as is becoming standard research practice.[68] For example, the pristine origin of the Poudre River in Colorado,[23] zones of Swiss lakes distal to known point sources,[26] and antibiotic-free livestock[44] are all points of "background" comparison that have been successfully applied to identify anthropogenic ARG sources.

The question still arises regarding *What* to compare to a given background? With qPCR, both absolute and relative levels of target ARGs can be directly compared. This approach has become standard practice and has revealed remarkable correlation, as noted previously, for both the *sul*1 ARG[23] and the *int*I1 Class 1 integron.[55] Similarly, with metagenomic approaches the relative abundances of ARG types and classes with higher "risk" rankings can be determined and compared. Comparisons of ARG and MGE diversities are also of interest, with the logic that greater variety in ARGs and MGEs result in increased opportunities for transfer to pathogens. The assembly of metagenomic data sets can now provide information regarding whether individual ARGs are located on MGEs as well as which bacteria host a given ARG or MGE.[69] As metagenomic sequencing becomes increasingly available while also providing more robust genetic information, these types of studies will provide profound insights into which environmental compartments and pathways present the greatest risk and should be targeted for mitigation.

MITIGATING ANTIBIOTIC RESISTANCE AS AN ENVIRONMENTAL CONTAMINANT

Given that ARGs and MGEs can transcend their hosts, mitigation strategies may best be focused at the gene level to minimize the risk of downstream uptake and propagation.[59] As noted previously, the key environmental focal points include

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sewage, wastewater, livestock manure, compost, lagoons, and antibiotic manufacturing facility effluents. Efforts to mitigate resistance dissemination should focus on minimizing ARG and ARB proliferation within these systems, while simultaneously ensuring that ARG and ARB fluxes are well understood.

Restricting antibiotic use for only essential purposes is one of the most widespread methods to mitigate resistance dissemination. The logic behind this approach is that decreased antibiotic loads will decrease selection pressures and thus reduce maintenance of ARGs carried by the host such that resistant strains attenuate over time.[70] In theory, such efforts will lead to reduced antibiotic residuals in livestock waste and within wastewater treatment plants, and should reduce the likelihood for gene transfer and selection within these compartments. One major challenge, however, is that countries across the world differ with respect to their antibiotic prescription and use practices, both for humans and for agriculture. Given the rapidity at which resistance spreads globally it is thus likely that the least-restrictive antibiotic use practices will play an outsize role in resistance development and dissemination. Finally, it is important to consider that there are a wide variety of potential selection agents. The importance of these other agents is well-known, but as of yet poorly understood, particularly under conditions where multiple selection agents are simultaneously present.

There is growing recognition of the logic of limiting ARG dissemination. "Prudent" mitigation practices could be formulated that, based on the literature, attenuate target ARGs and MGEs via approaches that are economical and in harmony with other environmental benefits.[17] In the context of wastewater treatment, it should be recognized that the disinfection practices used to control traditional pathogens or that are used for micropollutant removal, such as chlorination or ozonation, have the potential to select for antibiotic resistance[71,72] and are limited in their ability to destroy all of the genetic material in wastewater under most current operation conditions.[59,61,73,74] Thus, traditional pathogen mitigation methods will need to be adapted to address antibiotic resistance.

THE PATH FORWARD

There is now global recognition of the problem of antibiotic resistance and a growing body of research documenting how environmental sources and pathways contribute to its spread. A large amount of data has accumulated on the occurrence of ARGs in environmental niches that definitively demonstrate the influence of anthropogenic inputs. However, efforts are required to translate the results of these studies into strategic monitoring and mitigation efforts. Such a goal would be most effectively achieved if there are agreed upon targets and measures of antibiotic resistance in the environment, particularly those that distinguish human inputs from the background and that are most relevant to human and ecosystem health. Coordinated efforts on several fronts are needed to help identify which ARBs and ARGs to monitor and by which metrics they should be assessed.

First, meta-analysis of existing data could help identify key markers of resistance characteristic of specific environments (e.g., different types of livestock operations, domestic wastewater treatment plants, or hospitals). Such an effort would help identify the ARBs, ARGs, and MGEs that are most indicative of human influence. To take this a step further, efforts to backtrace particularly problematic ARGs observed in the clinic to their environmental sources would help identify critical control points that could be targeted. Open data access, ideally through public databases, is essential to achieving this goal, as is the quality and standardization of the data reported. For example, discussion of relevant background concentrations, meta-data, and appropriate statistical analysis must be included in monitoring studies.[75] Clearly, the quality of the scientific work will dictate progress in the development of coordinated strategies to combat antibiotic resistance.

Second, methods for characterizing ARGs in the environment are rapidly improving. Metagenomics has already provided an unprecedented depth of information within just a few years and is enabling comprehensive profiling of total ARGs. Newer technologies (e.g., PacBio, MinION) that enable collection of much longer DNA sequences have the capacity to more precisely identify ARGs and their adjacent genes. In this manner, they should provide useful information about the MGEs and bacterial hosts that harbor a given ARG.

Third, risk assessment models tailored to environmental sources and routes of antibiotic resistance dissemination are needed. However, developing and parametrizing the models will take a significant amount of time and effort. This is especially true when the question of *What* exactly to monitor remains unanswered. There thus needs to be coordination and feedback between the identification of monitoring targets and the development of risk models. As progress is made, risk models can help inform policy in terms of *Where* to target monitoring and mitigation and *Which* criteria and levels should be met.

Fourth, given the urgency of the problem of the spread of antibiotic resistance, the science of resistance mitigation must continue to move forward and be applied where practical. There is now a significant body of research documenting the fate of ARBs and ARGs through wastewater treatment plants, agricultural, and other systems that can be drawn upon to glean insight into which treatments and technologies are most likely effective. Mitigation practices are beginning to be adopted, particularly in situations where stakeholders want to be proactive to address greater perceived risk or strong public concern. Such proactive practices may make sense, particularly if any added costs can be kept to a minimum and there are additional benefits. Systematic and hypothesis-driven research is required to confirm treatment effectiveness and to inform practical best management practices. In particular, research providing insight into the mechanisms by which ARGs are selected and horizontally transferred is needed.

Finally, it must be recognized that antibiotic resistance does not respect regional or international borders. Global monitoring efforts will provide insight into how local policy, practice, and socioeconomic factors influence antibiotic resistance. International collaboration and data sharing is thus essential for defining baselines and mitigation end points to prevent and contain resistance in order to maintain antibiotics as a precious resource for future generations.

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■ GLOSSARY

ARB. antibiotic resistant bacteria. Bacteria that are resistant to one or more antibiotics

ARG. antibiotic resistance gene. Genes that encode for resistance to an antibiotic

***bla*$_{KPC-2}$ and *bla*$_{NDM-1}$.** antibiotic resistance genes that encode for production of carbapenemase enzymes. Carbapenemases are β-lactamases that hydrolyze most beta-lactam antibiotics, including carbapenems

DNA. deoxyribonucleic acid. A molecule that stores biological information. Each molecule contains a code based on the sequence of four bases (adenine, guanine, cytosine, thymine)

Gene. a region of DNA, the sequence of which informs specific functions in the cell, such as production of specific proteins

HGT. horizontal gene transfer. The sharing of genes between bacterial cells

***int*I1.** class 1 integron. Integrons are genetic elements that facilitate the integration, expression, and exchange of DNA. Specific amino acid sequences differentiate integrons into different classes

MDR. multidrug resistant. Bacteria that are simultaneously resistant to multiple different antibiotics

MGE. mobile genetic element. A DNA segment that encodes enzymes or other proteins that facilitate movement of genetic material between cells. Includes transposons, plasmids, integrons

QMRA. quantitative microbial risk assessment. Considers microbial behavior to identify where microbes can become a danger and then estimates the risks and uncertainties that they pose to human health

***qnr*S.** antibiotic resistance gene that encodes for resistance to fluoroquinolone

Resistome. the sum total of all of antibiotic resistance genes in a particular environmental niche

***sul*1.** A specific antibiotic resistance gene that encodes for resistance to sulfonamide antibiotics

tet. generic class of antibiotic resistance genes that encode for resistance to tetracycline. Specific *tet* genes include *tet*W, *tet*M, *tet*A, etc..

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