

An Environmental Science and Engineering Framework for Combating Antimicrobial Resistance

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Received: December 14, 2017

Accepted in revised form: April 6, 2018

Abstract

On June 20, 2017, members of the environmental engineering and science (EES) community convened at the Association of Environmental Engineering and Science Professors (AEESP) Biennial Conference for a workshop on antimicrobial resistance. With over 80 registered participants, discussion groups focused on the following topics: risk assessment, monitoring, wastewater treatment, agricultural systems, and synergies. In this study, we summarize the consensus among the workshop participants regarding the role of the EES community in understanding and mitigating the spread of antibiotic resistance via environmental pathways. Environmental scientists and engineers offer a unique and interdisciplinary perspective and expertise needed for engaging with other disciplines such as medicine, agriculture, and public health to effectively address important knowledge gaps with respect to the linkages between human activities, impacts to the environment, and human health risks. Recommendations that propose priorities for research within the EES community, as well as areas where interdisciplinary perspectives are needed, are highlighted. In particular, risk modeling and assessment, monitoring, and mass balance modeling can aid in the identification of “hot spots” for antibiotic resistance evolution and dissemination, and can help identify effective targets for mitigation. Such information will be essential for the development of an informed and effective policy aimed at preserving and protecting the efficacy of antibiotics for future generations.

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Keywords: biological systems; environmental microbiology; molecular biology; monitoring methods; risk

Key Knowledge Gaps for Antibiotic Resistance in the Environment and Research Needs

MASS PRODUCTION AND USE OF ANTIMICROBIALS, especially antibiotics aimed specifically at treating and preventing bacterial infections, represent a two-edged sword. They provide revolutionary public health benefits, but overuse is linked to diminishing returns because of the potential to select for resistant strains. The growing global threat of antibiotic resistance to human health is widely acknowledged. The following subsections summarize the key knowledge gaps and research needs for better understanding and mitigating environmental sources and pathways of antibiotic resistance in working toward preserving the utility of these life-saving drugs.

Risk modeling and assessment

Substantial advances have recently been made in the field of quantitative microbial risk assessment (QMRA). These advances provide a framework for determining concentrations of pathogens via various environmental exposure routes that are capable of causing illness (Haas *et al.*, 2014; Haas, 2015). However, there is an urgent need to extend the QMRA concept in a way that captures the environmental dimension of antibiotic resistance (Ashbolt *et al.*, 2013). Progress toward risk assessment is essential to link human exposure to antibiotic-resistant bacteria (ARBs) able to infect humans and antibiotic resistance genes (ARGs) present in the environment or excreted by domestic animals or humans.

Identifying critical risk thresholds for ARB and ARG exposures through food, water, and air that influence human health will be particularly key to develop mitigation strategies (Vikesland *et al.*, 2017). For traditional pathogens, such information can be derived from dose–response studies; however, antibiotic resistance requires a different paradigm. For example, instances of multiple exposures to bacteria carrying ARGs, asymptomatic colonization that may be followed by delayed onset of illness with an AMR organism, multiple interacting/competing strains of susceptible/resistant pathogens, and horizontal gene transfer in the environment and/or host complicate the traditional dose–response paradigm (Wassenaar *et al.*, 2007; Evans *et al.*, 2009; Garner *et al.*, 2016) and are likely to be important and challenging for risk assessment. While the most meaningful monitoring targets are yet to be identified, methods that provide quantitative measures of exposure and health effects will provide the greatest value from a risk characterization and assessment standpoint. Epidemiological studies that document the extent of acquisition of AMR organisms from environmental exposures or that quantify the acquisition of such bacteria from human or animal dose–response studies would be of value.

As a first step, a source, fate, and transport-based framework are logical means to capture the complexity of antibiotic resistance movement in the environment and to identify human exposure “hot spots” as well as points of targeted intervention. Such a framework, where animals, humans, and the “environment” represent three reservoirs or “stocks” of antibiotic resistance between which there are bidirectional

transports or “flows” (Fig. 1), could be adapted from prior work (Haas, 1983, 2000; Rose and Haas, 1999; Pujol *et al.*, 2009; Haas *et al.*, 2014). Example flows between compartments include pharmaceutical wastewater discharges to receiving waters, carriage of pathogenic bacteria or other biota carrying ARGs on meat or produce, classic exposure routes to human and animal excreta through air, water, and soil, irrigation with contaminated water, and application of manure or biosolid-derived soil amendments. The configuration and relative impact of each of these compartments may differ substantially in more developed and resource-rich countries where there is more rigorous implementation and enforcement of sanitation barriers, in contrast to countries where barriers between anthropogenic outputs and the environment as well as other sanitation strategies may be limited or nonexistent.

Complex dynamics within each reservoir, largely governed by microbial ecological processes, determines the prevailing levels of resistance. For example, the physicochemical conditions (e.g., concentration of antimicrobials, metals, or other stressors) and biotic factors (e.g., presence of mobile genetic elements [MGEs]) may interact in the selection of resistant versus susceptible strains. Importantly, in the absence of antibiotics, the actual competitive advantage of resistant strains relative to susceptible strains is currently unclear. In this context, the perspective from which the environmental engineering and science (EES) community defines “antimicrobial resistance” may play a role in the resolution at which risk is defined. For example, defining antibiotic resistance from a broad perspective (e.g., as one composite contaminant) could aid in identifying key environmental hot spots as well as in comparing their relative significance in terms of overall risk. On the contrary, a deeper look that separates ARGs into health risk classifications, such as those proposed by Martínez *et al.* (2015), within an environmental context, could yield higher resolution models that lead to more specific recommendations.

Coupling environmental monitoring of exposure sources with human epidemiological studies, for example, studies involving livestock farm workers and wastewater treatment plant (WWTP) operators (Rosenberg Goldstein *et al.*, 2014), could be particularly informative toward advancing exposure monitoring, health effects analysis, and risk assessment. Risk assessment coupled with surveillance monitoring could guide the development of fruitful strategies for mitigating the spread of antibiotic resistance (Pruden *et al.*, 2013). For example, modeling and sensitivity analysis can help identify which parameters are most influential, and thus, the most promising targets for control in environmental systems.

Surveillance monitoring

International institutions such as the World Health Organization (WHO), the European Union health organizations (EU Commission, 2017) and the nations adhering to the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR, 2017), strongly advocate the need for increased and coordinated monitoring of antibiotic resistance (WHO, 2014a, 2015; Wuijts *et al.*, 2017). Monitoring characterizes the landscape of antibiotic resistance, identifies key routes of

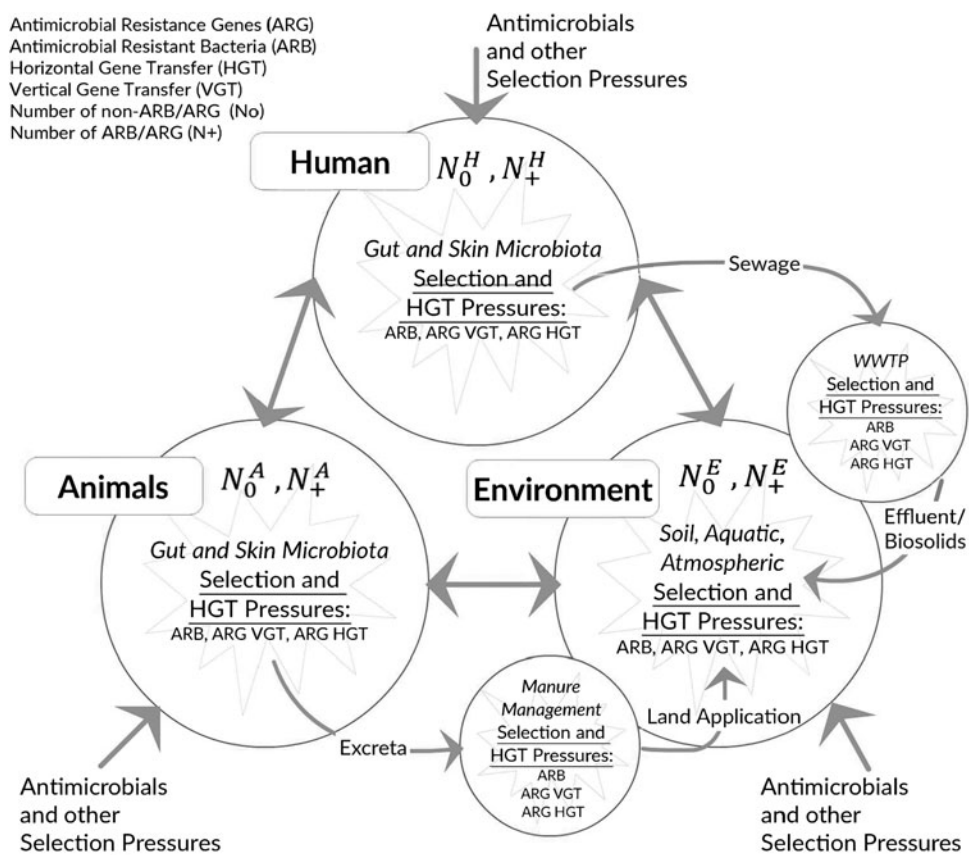


FIG. 1. Stocks and Flows Framework Conceptualizing the Movement of Antimicrobial Resistance in the Environment. N_0 is defined as the total number of bacteria sensitive to antibiotics and other selective pressures. N_+ is defined as the total number of bacteria resistant to antibiotics and other selective pressures. N_0 and N_+ are a function of growth rates (i.e., μ_0, μ_+) to be applied in fate and transport models.

exposure, and informs where to most effectively place barriers to the spread of resistance. However, the lack of agreed-upon environmental monitoring targets is a major challenge. One potential approach is to adapt standard fecal bacterial monitoring, which forms much of the global regulatory fabric, but focus specifically on resistant strains and genes of concern. For example, the WHO, the EU, and select countries in Asia and Africa have recently initiated a pilot surveillance program targeting extended-spectrum beta lactamase (ESBL)-producing *Escherichia coli* (Matheu *et al.*, 2017). The spread of ESBL is of utmost concern as a human health threat, whereas monitoring capabilities for *E. coli* are readily available and accessible worldwide. Other culture-based targets are also worthy of consideration, and it may be advisable at a minimum to culture representative gram-positive and gram-negative indicator bacteria, since antibiotic classes generally vary in their effectiveness toward these two groups. Culturing verifies the viability of target organisms and standardized assays (e.g., CLSI) and can be used to classify resistant strains (McLain *et al.*, 2016). However, viable but nonculturable strains, similarly important to human health risks, will not be captured.

It is important to recognize that culturing misses the majority of bacteria and fails to fully capture the ecological processes driving the dynamics within and between the various reservoirs of resistance (Fig. 1). In this study, nucleic acid-based methods such as next-generation DNA sequencing, metagenomics, and quantitative polymerase chain reaction (qPCR), provide the capacity for monitoring ARG movement within and between environmental compartments. In addition, molecular monitoring can provide data to iden-

tify the mechanisms contributing to the spread of antibiotic resistance, such as selection pressures, horizontal gene transfer, or transport of bacteria carrying ARGs. Knowing such mechanisms can help inform risk assessments and lead to more effective mitigation (Luby *et al.*, 2016).

Even as the majority of ARGs and MGEs can be captured with emerging metagenomic capabilities (i.e., the resistome) (Wright, 2007), it remains necessary to accurately identify them and appropriately analyze and interpret the data (Bengtsson-Palme *et al.*, 2017; Lakin *et al.*, 2017). Analogous to the fecal indicator paradigm, it would be useful to identify meaningful ARG and/or MGE targets indicative of AMR potential that could be subject to standardized monitoring. When selecting suitable ARG targets, important considerations include whether the form of resistance an ARG encodes is relevant to human health, if the ARG targets are associated with MGEs and have the capability to spread among different types of bacteria, if the ARG is present inside a human pathogen, and if the ARG is functional or deactivated (e.g., following disinfection) (Martínez *et al.*, 2015).

Importantly, even after cellular deactivation, extracellular DNA may remain functional with the potential to be taken up by other bacteria via transformation (Allen *et al.*, 2010; Dodd, 2012). Furthermore, it is essential that the EES community move toward standards in reporting, such as the types and methods of DNA extraction used and any QA/QC, detection limits, specificity, providing data in both normalized and un-normalized formats, sequencing depths, and criteria and cutoffs for identifying an ARG or MGE. Studies must be reported in a manner that is repeatable. In addition,

TABLE 1. RECOMMENDED PRIORITIES FOR RESEARCH AND ACTION AMONG THE EES COMMUNITY

| <i>General area in need of action</i> | <i>Recommended priorities for research and/or action</i> |
|--|---|
| Surveillance and monitoring | Standardization of methods and QA/QC practices Agreement on targets for monitoring Agreement on definition of antibiotic resistance in an environmental context Curation and structuring of antibiotic resistance databases to include ARG risk classification Standardization of data reporting (and enforcement within EES community) |
| AMR characterization within environmental hot spots (e.g., WWTPs, farms, pharmaceutical effluents) | Characterization of mechanisms of selection, transfer, and propagation of antibiotic resistance Identification of effective treatment technologies for minimization of AR outputs into receiving environments Expansion of international studies that provide insight into broader phenomena, including measurable effects of various antibiotic regulation and control strategies, hygiene and sanitation conditions, water and agricultural infrastructure, and socioeconomic factors |
| Risk modeling and assessment | Extension of QMRA to capture environmental dimension of antibiotic resistance Expansion of interdisciplinary system-level studies that focus on risk characterization Modeling of antibiotic resistance fate and transport and/or mass balances Identification of links between environmental exposure and epidemiological data Quantify links between degree of sanitation implementation and associated antibiotic resistance risks |

QMRA, quantitative microbial risk assessment; WWTPs, wastewater treatment plants.

metagenomic methods need to be improved to obtain absolute quantification, rather than just relative abundance.

Wastewater treatment

WWTPs, given their high bacterial densities and that they receive antibiotics, metals, and other selective agents, are a logical focal point of the EES community. Numerous studies have now profiled and tracked ARGs and ARBs through municipal WWTPs, as well as the impacts of effluent and biosolids on their receiving environments, but there is a need for translation of findings into practical, meaningful, and actionable guidance for WWTP designers and operators. Future studies should inform quantitative estimates of relative loadings from solid versus liquid product streams as a guide for where to focus attention for mitigation (Munir *et al.*, 2011) and to provide context with respect to WWTP-associated loadings relative to other potential sources. A key challenge is to identify if there are critical AMR and/or selective agent loading rates that may lead to measurable concentration increases or decreases, to inform possible control targets. Also, it is essential for wastewater management strategies to be relevant to local conditions. For example, cost-effective and low-technology solutions are particularly critical in lesser developed countries. Ensuring basic sanitation as the primary barrier for AMR spread should be the first and foremost goal of any antibiotic resistance management action plan (Laxminarayan, 2014; Andremont and Walsh, 2015).

A clear quantitative analysis of the ecological processes occurring within biological treatment systems is needed to assess their net contribution toward attenuating (e.g., reducing fitness of resistance strains) or propagating (e.g., providing an

environment ideal for selection and horizontal gene transfer) antibiotic resistance. Furthermore, moving beyond observational studies toward more controlled, mechanistic studies that may better identify factors that promote or diminish antibiotic resistance transfer during biological treatment is necessary. Of interest are the relative roles of antibiotics, metals, virulence factors, and resistant bacteria and pathogens in the influent and effluent, versus operational conditions such as suspended or attached growth, aerobic versus anaerobic, mesophilic versus thermophilic, solids retention time, and hydraulic residence times (Diehl and LaPara, 2010; Zhang and Li, 2011; Bengtsson-Palme *et al.*, 2016; Neyestani *et al.*, 2017a, 2017b). Studies that address physiology, ecology, and processes for managing the microbial ecology of AMR during wastewater treatment are needed, along with prioritization of waste streams with high potential to spread AMR, such as those from antibiotic production processes and hospitals.

A multibarrier approach to wastewater treatment may be most advisable to minimize the impacts of ARBs and ARGs from the application of residual biosolids as a soil amendment and in wastewater reuse. For biosolids, hydrolytic treatments, anaerobic digestion (particularly thermophilic), and composting are potential options (Diehl and LaPara, 2010; Zhang and Li, 2011; Bengtsson-Palme *et al.*, 2016). Ideally, effluent treatment strategies should disinfect and destroy ARGs and remove potential selecting agents such as residual antibiotics or metals (Dodd, 2012). For the final effluent, membrane-based treatments will likely offer the most stringent treatment. Although several studies have addressed the efficacy of disinfection strategies against antibiotic resistance (McKinney and Pruden, 2012; Czekalski *et al.*, 2016; Chang *et al.*, 2017), currently, surprisingly little is

known about potential unintended effects of various disinfectants, including chlorine, chloramines, ozone, and other advanced oxidation processes, other than the possibility that they select for more resistant (and multidrug-resistant) strains (Luo *et al.*, 2014; Guo *et al.*, 2015). Some studies have identified circumstances that select for resistant strains, which then have the opportunity to preferentially regrow downstream (Xi *et al.*, 2009). This observation highlights the need to consider monitoring environmental pathogens (e.g., *Aeromonas* or *Arcobacter* spp.) that grow during and following treatment (Banting *et al.*, 2016; Rasmussen-Ivey *et al.*, 2016). Overall, mitigation strategies must be harmonized with the need for water sustainability and reuse, because the societal need for water overrides the possible risks of antibiotic resistance.

Agriculture

Antibiotics are widely used in the livestock industry, more so on a mass basis than in humans (Van Boeckel *et al.*, 2015). The EU phased out subtherapeutic use of antibiotics over a decade ago, while the United States only recently followed suit (December 2016) with the Veterinary Feed Directive requiring a prescription for antibiotic use in livestock and prohibiting the use of antibiotics as growth promoters (FDA, 2012, 2013, 2017). More recently, in 2017, the WHO published its “Guidelines on use of medically important antimicrobials in food-producing animals” following the 5th revised list of “critically important antimicrobials for human medicine” published in 2014 (WHO, 2014b, 2017). An interdisciplinary system perspective along the full “farm to fork” continuum is recommended to investigate the impacts of these policies.

Collaborating with and learning from the food and agricultural sciences can aid in identifying promising on-farm mitigation opportunities, including manure management, pre- and postharvest practices, and meat processing and packing. With this approach, there are clear synergies with current calls to advance the efficiency of food–energy–water systems. System complexity and nonpoint contributions are major challenges to address. As with wastewater treatment, multiple barriers are advisable (O’Neill *et al.*, 2015), especially given the complexity of nonpoint source mitigation. In contrast to the management and treatment of municipal wastewater, food animal waste management has no regulated fecal microbial reduction requirements in the United States and most other countries. Developing effective and economical approaches to minimize ARBs and ARGs in animal manure is a critical challenge.

Key issues in recommending mitigation strategies for agricultural sources of antibiotic resistance include the following. Are current guidelines and policies effectively implemented in practice and spirit, if so, are they working beneficially with respect to both animal health and environmental conservation, and what are the synergies with other drivers such as consumer preference and the “organic” movement? There is an opportunity to learn from Europe and other countries regarding successful interventions. Furthermore, attenuation of resistance may require decades to be observable, particularly if resistant bacteria have acquired a permanent niche within the livestock microbiome. Environmental modeling could help predict the effects of changes in antibiotic use policies and mitigation options. Recognizing that farmers are typically operating with marginal profits

makes it critical that recommendations do not put people out of business and hurt the food supply in years to come, especially as population growth and food demands become increasingly acute.

Recommendations

Environmental scientists and engineers provide a unique and valuable perspective and tool set toward understanding and addressing antibiotic resistance. Recommendations regarding priority areas where the EES community may contribute toward countering the spread of antibiotic resistance via environmental pathways are highlighted in Table 1. In particular, large interdisciplinary system-level studies that focus on risk characterization, especially those grounded in real-world conditions and practice, can provide AMR monitoring and yield recommendations for mitigation strategies in environmental systems. Such studies could ideally be coupled with fate and transport/mass balance modeling, using a core analytical system framework that is well established in the EES community.

In particular, hypothesis-driven environmental research that provides insight into mechanisms of selection, transfer, and propagation of antibiotic resistance and its linkage to human health risk assessments are needed. Development of risk assessment models specifically tailored to the environmental roles of antibiotic resistance was identified as a top priority, especially given the need for actionable endpoints for both monitoring and mitigation. International studies are also of value, providing insight into broader phenomena, including measurable effects of various antibiotic regulation and control strategies, hygiene and sanitation conditions, water and agricultural infrastructure, and socioeconomic factors. There is a need for rigorous QA/QC in data collection, as well as agreement in the community regarding standardized methods and reporting. Until priority monitoring targets are agreed on, analysis of a suite of culture-based and molecular-based indicators is logical, as is making raw data and unit analysis publicly available. Such criteria should be considered in the peer-reviewed evaluation of literature.

Collectively, contribution from the EES community is essential to inform effective practices and policies for combatting antibiotic resistance toward preserving and protecting antibiotics for continued benefit and protection of public health in the future.

Acknowledgments

The authors thank and acknowledge all the participants in the Slowing the Spread of Antimicrobial Resistance via Environmental Pathways: Risk Assessment and Management Perspective Workshop that was held at the Association of Environmental Engineering and Science Professors (AEESP) Biennial Conference, June 20, 2017, University of Michigan, Ann Arbor, MI, as well as the National Science Foundation Partnership in International Research and Education Award 1545756. Participants representing each of the subgroup topics volunteered to synthesize the discussions and contribute as coauthors to this article.

Author Disclosure Statement

No competing financial interests exist.

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