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Seizing the moment: now is the time for integrated global surveillance of antimicrobial resistance in wastewater environments

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Antimicrobial resistance (AMR) is a growing global health threat that requires coordinated action across One Health sectors (humans, animals, environment) to stem its spread. Environmental surveillance of AMR is largely behind the curve in current One Health surveillance programs, but recent momentum in the establishment of infrastructure for monitoring of the SARS-CoV-2 virus in sewage provides an impetus for analogous AMR monitoring. Simultaneous advances in research have identified striking trends in various AMR measures in wastewater and other impacted environments across global transects. Methodologies for tracking AMR, including metagenomics, are rapidly advancing, but need to be standardized and made modular for access by LMICs, while also developing systems for sample archiving and data sharing. Such efforts will help optimize effective global AMR policy.

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Introduction

Antimicrobial resistance (AMR) is a looming global public health threat that increasingly undermines treatment options for deadly infections. Most recent estimates place the health burden of antibiotic-resistant bacterial infections to be comparable to influenza, tuberculosis, and HIV/AIDS combined [1], and this is likely an underestimation [2]. The COVID-19 pandemic has made it abundantly clear that microbes do not respect borders and are readily dispersed via commerce, travel, and various earth system processes. Tracking the global dissemination of AMR is particularly daunting because there is no single agent responsible. In contrast to SARS-CoV-2 or influenza, there are numerous species of antibiotic-resistant bacteria (ARB) of concern. While pathogenic strains present immediate acute health risks, non-pathogenic ARB are active reservoirs of antibiotic resistance genes (ARGs) that can be horizontally transferred to pathogens. It is currently understood that most ARGs and virulence genes were actually acquired by human pathogens from environmental bacteria via horizontal gene transfer [3•]. Furthermore, beyond bacteria, there is growing worry about the broader resistance of other microbes, for example, to antiviral and antifungal treatments [4]. Recognizing the importance of antimicrobials to both human and animal health, as well as the need to holistically consider the movement of AMR among people, animals, and the environment, the need for a One Health framework for understanding, tracking, and mitigating the problem is broadly gaining acceptance [5•,6,7•,8].

Surveillance is a fundamental tenet of global public health protection, and various national and international systems are in place for tracking infections caused by reportable pathogens (e.g. *Legionella* in the US, EU, and Australia [9], Ebola virus globally [10]). In the context of AMR, most surveillance has historically targeted clinical and food sectors, with a focus on faecal indicators and the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) pathogens. In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) serves to compare enteric ARBs isolated from food production systems to clinical strains while the recently launched European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-vet) aims to assess the risk of AMR transmission between diseased animals and humans via non-food-related routes [11•]. Comparable environmental monitoring that fully informs the One Health framework has been lacking (Figure B1). The confluence of air, water, soil, and the waste streams (domestic, agricultural,

Figure B1

Growing Infrastructure for Environmental AMR Surveillance

- World Health Organization Tricycle Program
- Joint Programming Initiative on Antimicrobial Resistance (JPIAMR): Establishing a Monitoring Baseline for Antibiotic Resistance in Key environments (EMBARK)
- National Antimicrobial Resistance Monitoring System (NARMS): Surface Water AMR Monitoring (SWAM)
- Global Sewage Surveillance Project
- Global Antimicrobial Resistance Surveillance System (GLASS)
- European Antimicrobial Resistance Surveillance Network (EARS-net)
- European Antimicrobial Resistance Surveillance Network in Veterinary Medicine (EARS-vet)
- Tripartite Integrated System for Surveillance on AMR and Antimicrobial Use (TISSA)

What is Missing?

- Full integration of human, animal, and environmental AMR monitoring data
- Centralized data accessibility and transparency with robust and informative metadata
- Standardized culture- and molecular-based AMR monitoring
- Integration of multiple types of AMR monitoring data and metadata
- Risk assessment frameworks appropriate for environmental AMR and adaptable to different data types and questions

Opportunities and Benefits for Environmental AMR Monitoring

- Holistically and more accurately define the drivers and environmental burden of AMR
- Rapidly identify hotspots for evolution and spread of clinically-relevant AMR determinants
- Provide large, longitudinal datasets for identifying drivers of AMR and predicting trends
- Identifying epidemiological links between the environment, humans, and animals
- Inform human and animal clinicians about which antibiotics will be most effective at population-specific scales
- Providing data needed to inform risk assessment models and target regulatory limits
- Identify treatment technologies that most effectively mitigate AMR spread in both HICs and LMICs
- Data-driven policy action and the investment of resources to combat global AMR

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Existing infrastructure that can be expanded upon for coordinated international environmental AMR surveillance, including gaps and opportunities, to inform and refine policy for combatting AMR.

and industrial) that flow into and between them, are understood to contain key hotspots for the evolution and transmission of ARGs in particular and AMR in general [12••,13•,14].

Establishing coordinated local, regional, and international surveillance systems for tracking environmental AMR presents numerous advantages and opportunities towards shaping antimicrobial use policy and practice in a manner that will ensure their efficacy for future generations [15••]. While there has been inertia towards environmental monitoring of AMR, recent developments, including the broad infrastructure emplaced for SARS-CoV-2 surveillance in wastewater over the past year [16], present a tipping point towards actualization. However, it is also critical at this moment to strategically plan and coordinate such efforts to maximize potential benefits.

Wastewater surveillance: history, COVID-19, and synergy for advancing AMR surveillance

In 1929, Gray published on the isolation of *Bacillus paratyphosus* B (i.e. *Salmonella enterica* serotypes (Paratyphi A–C)) from sewage concluding that the method could be adopted for tracing of carriers [17]. Later, wastewater surveillance was employed for many pathogens including viruses [18]. In 2003, WHO embraced environmental surveillance of poliovirus to support poliovirus eradication efforts, targeting locales with low vaccination coverage and emergence [19], as was shown for the Netherlands [20]. The sensitivity of sewage surveillance was estimated to be 100 infected (asymptomatic) individuals in >10 000 individuals in a poliovirus vaccine field study [21]. With the onset of the COVID-19 pandemic, there was spontaneous recognition of the value that sewage monitoring of the virus and corresponding research and infrastructure was rapidly established across numerous universities and wastewater treatment plants (WWTPs) around the world [22,23]. Widescale surveillance of SARS-COV-2 has brought to light numerous advantages, as a sensitive, early warning tool to aid public health decision making [24].

The rapid development of infrastructure for SARS-COV-2 monitoring is remarkably timely with respect to recent momentum in research aimed at sewage surveillance of AMR. A study in Hawaii demonstrated that *Salmonella* detected in sewage could be traced to ongoing unreported foodborne outbreaks, with whole genome sequencing (WGS) applied to obtain a high-resolution resistance profile [25••]. The application of shotgun metagenomic sequencing to sewage has further recently revealed striking concordance of ARG abundances with national stringency in antibiotic use policy and socioeconomic factors likely contributing to the spread of resistance [26••,27•,28]. Sewage surveillance is particularly advantageous because it provides an integrated view of AMR carried across a human population, which is much more efficient than the current *status quo* of patient-by-patient sampling and also avoids privacy concerns in the collection of data [29••]. Approaches currently in development can also easily be extended to environments collectively influenced by agricultural inputs, such as waste lagoons and surface waters [30], making for the possibility of a true One Health monitoring system. For example, efforts are currently underway to expand the traditionally retail meat and food-producing animal focused NARMS to include surface water, as an integrator of domestic, industrial, and agricultural inputs [31••]. Thus, the present moment is ideal to extend the tremendous resources that have been invested towards pandemic monitoring of sewage towards environmental surveillance of AMR.

Environmental surveillance of AMR: need for adaptable, modular, and standardized methods

In order for the full benefits of environmental AMR monitoring to be realized, a coordinated approach will be essential to ensure that the data are comparable and actionable. To achieve this, standardized methods are needed (Figure 2). Grab, passive, or composite (flow-weighted or time-weighted) samples [32] can be collected either from the influent to a WWTP or further up the sewage collection system. Discrete locations within the sewer system can provide more granular (i.e. building-level or street-level) information and isolate sensitive populations, such as hospitals, nursing homes, as well as potential industrial inputs, such as pharmaceutical manufacturing plants. Collection of available metadata (e.g. WWTP/lagoon capacity, flow rate, time temperature, pH) are essential to interpreting the data.

Given the complexity of AMR and the contributing factors, there are numerous analytical approaches, all providing insight into the parts of the 'elephant of AMR', but not the whole picture [33,34•] (Figure 2). Culture of relevant faecal indicators or pathogens provides a direct measure of viable ARBs of concern, which can be enumerated via plate counts and subsequently subject to further phenotypic profiling of various forms of AMR and/or WGS for high resolution genotypic profiling. Recently, the WHO has launched a standardized Tricycle Protocol for enumerating extended-spectrum beta lactamase-producing (ESBL) *Escherichia coli* intended to be suitable across the One Health spectrum [35••]. Similar protocols have been developed to more broadly capture resistant Enterobacteriaceae, targeting ESBL [36] and carbapenem-resistant forms [37•]. Culture is attractive as an entry point for LMIC participation, as faecal indicator monitoring infrastructure is relatively low cost and widespread. Isolates can be sent to centralized labs where resources can be effectively pooled, for example, through the WHO, to support archive and further characterization via WGS or phenotypic testing.

Molecular monitoring also presents several advantages and can also be adapted to LMIC settings, with the minimum entry point being the extraction of DNA. DNA can then be subject to a quantitative polymerase chain reaction (qPCR), providing highly sensitive quantitation of specific targets of concern, such as specific clinically relevant ARGs (e.g. ESBL-producing ARGs). qPCR quantification of anthropogenic indicators of AMR, such as *sul*1 and class 1 integrons [38], can also be quite informative with respect to evaluating the tendency of water treatment processes to reduce AMR and the overall persistence of AMR in affected receiving environments. Elevated anthropogenic indicators can also flag potential hot spots, for example, those receiving high loads of ARGs and/or where selection pressures are high, for deeper study with other methods. A drawback to all molecular-based measures is an inability to confirm the viability of targets.

DNA extracts could also be sent to centralized labs for shotgun metagenomic sequencing, which presents the advantage of being able to broadly capture the dominant genes representing the microbial community of a given environmental sample, without *a priori* selection of targets [39]. Resulting metagenomes can be compared to public databases, such as the Comprehensive Antibiotic Resistance Database (CARD) [40•], to profile ARGs belonging to various classes of interest (currently >3000 curated ARGs). Metagenomics also presents the advantage of providing rich contextual information, including taxonomic profiles of the microbial community and profiles of mobile genetic elements (MGEs), such as plasmids, integrons, and transposons known to play a key role in the dissemination of ARGs [41•,42,43].

Metagenomic data can further be searched for other pathogens of interest, such as fungi and viruses, although sample processing may need to be adjusted, for example, capture of smaller particles and extraction of RNA in addition to DNA. DNA sequencing, including metagenomics and WGS, is also really the only currently available approach to detecting newly emerged/emerging ARGs of concern. The present downsides of metagenomics include cost, high detection limits for highly diverse samples (e.g. environmental samples), the need to standardize bioinformatic approaches to ensure comparability of data, and general specialized expertise required to interpret the data that is likely lacking at the local level. These challenges can be addressed, in part, through centralized sample archiving, processing, and data analysis, which can additionally be supported through coordinated financing, to ensure access by LMICs [29••].

Although metagenomics is an emerging tool for AMR surveillance, it is clearly very powerful and already enabling remarkable discoveries. For example, recent comparisons of sewage collected across global transects have revealed that geographical location and socioeconomics are stronger predictors of elevated sewage ARG abundances than available antibiotic use data [26••,28]. Longitudinal metagenomic monitoring of Hong Kong WWTPs further showed that the activated sludge ARG composition shifts slowly over the span of nine years [44] and of a small US WWTP demonstrated that effluent metagenomes retain clinically relevant resistance patterns with strong concordance to clinical monitoring data [45•].

Building on growing AMR surveillance infrastructure

Substantial momentum has been gathered in recent years that provide the valuable groundwork for environmental AMR monitoring. In particular, the WHO Global Action Plan (GAP) (2015) and the EU One Health Action Plan against AMR [5•] have been highly influential in setting the stage. By 2017, the WHO estimated that roughly 2/3 of the World Health Assembly Member States had completed National Action Plans for combatting AMR, encompassing approximately 85% of the world's population [46,47]. Of the five strategic objectives in the WHO GAP, at least three collectively address concerns across human, veterinary, and environmental domains by improving awareness and understanding, strengthening knowledge through surveillance and research, and reducing the incidence of infection. However, there is a need for relevant environmental dimensions of AMR to be much more explicit, especially with respect to need for corresponding surveillance. The Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), a global collaborative currently of 28 nations, is a major step in the direction needed for global One Health AMR surveillance with a robust environmental dimension, but many major industrialized nations have not yet joined. The Tripartite Integrated System for Surveillance on AMR and Antimicrobial Use (TISSA) is in development at this time and could be an ideal mechanism to achieve the kind of global participation and coordination across nations that is urgently needed to realize the benefits of environmental AMR surveillance (Figure B1).

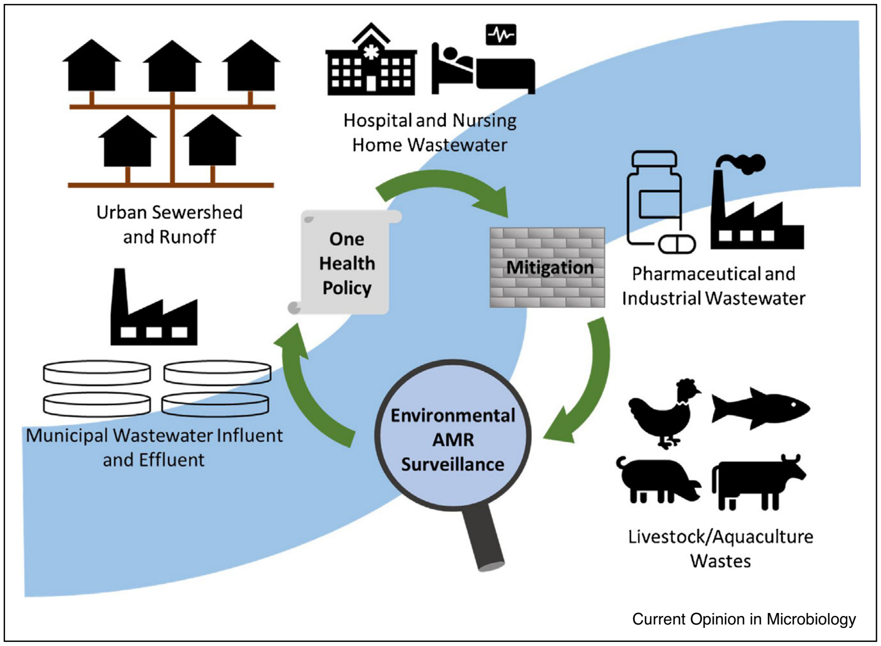
Global environmental surveillance of AMR and data sharing to inform policy and practice

It has become abundantly clear that the *status quo* of piecemeal monitoring in clinical and food productions systems will not be sufficient to stem the wicked problem of AMR. Environmental surveillance can help to provide essential information needed to efficiently and effectively inform policy and practice across the One Health spectrum to help keep antibiotics working for future generations. At the same time, surveillance can serve to assess the efficacy of various policy and mitigation measures, while also informing refinement and optimization with time (Figure 1) [48••]. Figure B1 summarizes a growing body of infrastructure that can be leveraged to advance environmental AMR monitoring, identifying key gaps and opportunities.

The first key opportunity that AMR surveillance of wastewater and corresponding receiving environments would bring would be the ability to rapidly identify hotspots for the evolution and spread of clinically relevant AMR. This would be especially valuable for guiding policy action and the investment of resources where they are most needed. For example, if pharmaceutical or hospital discharges are found to present high levels of mobile clinically relevant ARGs and pathogens, then it would be logical to regulate these waste streams in a manner appropriate to the individual country and local situation. Metagenomic approaches may be particularly suitable for this purpose [29••], aiding in the broad detection of novel ARGs and/or ARGs that have not previously been detected in a given location [49]. The detection of newly emerging or recently mobilized ARGs that have not yet become fixed in a microbial population may be limited though by the relatively high limits of detection of shotgun sequencing of complex environmental samples [50].

The second key opportunity that such environmental surveillance would bring would be to inform doctors and veterinarians with respect to which antibiotics are likely to be most effective within a given population. Centralized sewage surveillance can contain signals that reflect community-level dissemination of AMR. Similar to SARS-CoV-2, AMR may be silently transmitted within communities and missed by standard clinical surveillance [25••]. Such an approach is currently being piloted in the Netherlands, where culture-based monitoring of CPE

Figure 1



Informing One Health policy towards mitigating AMR through environmental monitoring. Surveillance informs policy aimed at mitigating the spread of AMR in human and agricultural systems and also serves to evaluate mitigation efforts to further develop policy and improve efficacy of mitigation efforts.

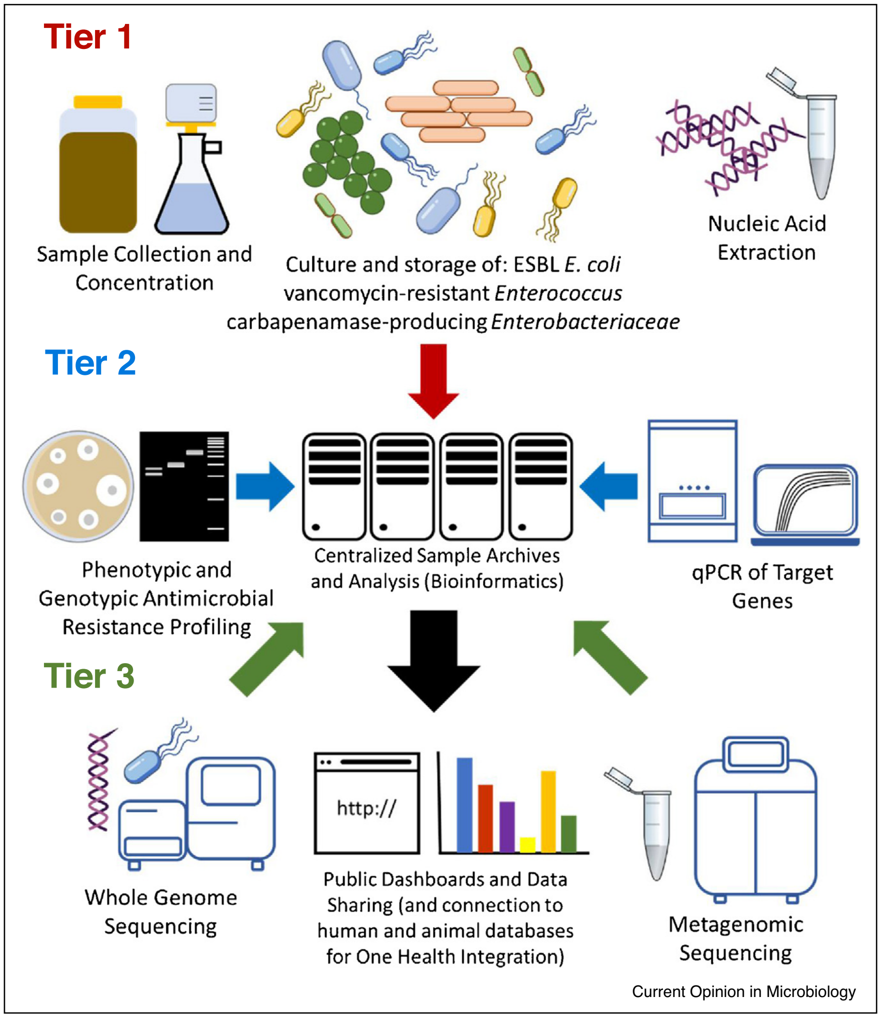
and subsequent WGS is helping to link to infections in the clinic and strains found in agricultural monitoring [37•]. The recently launched WHO Tricycle protocol for standardized ESBL *E. coli* enumeration in environmental samples [51] is poised to play a leading role in informing clinical and agricultural practice. Data compatibility and sharing will be essential to the success of such endeavours. Publicly facing dashboards, such as those popularized during the current COVID-19 pandemic, would be ideal for achieving this purpose [52]. Importantly, environmental and public health monitoring agencies are now working much more closely with local clinicians, many of whom even obtained their own PCR units for COVID testing that could potentially be adapted to ARG testing. Ideally, this can also help improve clinical AMR reporting, which is known as a longstanding lost opportunity as clinics primarily act only to treat patients and not to participate in research.

The third key opportunity of environmental surveillance would be to inform risk assessments, which is critical to guiding regulatory limits for the discharge of antibiotics, ARBs, and ARGs. There are various approaches to achieving this with the general recognition of the need to broaden and adapt traditional frameworks [53•,54], such as quantitative microbial risk assessment. One key challenge is differentiating human health risk, that is, estimating probability of infection based on a given exposure, from the risk of contributing to the evolution and spread of AMR. In both cases, the ability of ARGs to be horizontally transferred among bacteria is a critical phenomenon to account for [53•]. Culture-based approaches are more amenable to traditional human health risk assessments, whereas other algorithms are being explored to assess general risk of spread of clinically relevant ARGs to new pathogen hosts [55,56•] and to model and predict the various environmental processes (e.g. in a WWTP) that can contribute to the selection, amplification, horizontal transfer or attenuation of ARGs (e.g. Bayesian analysis [57,58]).

The fourth key opportunity would be to identify waste treatment technologies that are most effective for mitigating the spread of AMR. Wastewater and on-farm treatments have been an active area of research that can be tapped into to guide treatment practices, both in HICs and LMICs, as summarized comprehensively by Ashbolt *et al.* [59•]. Centralizing the accessibility and amenability of such data for analysis would be a tremendous resource towards guiding engineers and farmers in the selection of appropriate treatment processes and best management practices for stemming the spread of AMR.

Finally, environmental surveillance of AMR would provide vital missing data need to support large-scale modelling to identify key drivers. In particular,

Figure 2



A tiered approach to coordinated and standardized environmental AMR monitoring. Tier 1 is most accessible and should be carried out by all participating locales. Tier 2 may be carried out in-house or by centralized facilities. Tier 3 is likely to be carried out by centralized facilities and will be least accessible due to cost, but cost and accessibility is expected to improve in coming decades. Centralized sample archives and public-facing dashboards facilitate standardization of data analysis and reporting and data sharing. Sample archives will further provide value for future re-analysis as technology evolves.

machine-learning based approaches could be extremely powerful towards achieving all of the above objectives [60], detecting emerging ARGs and variants [61], in addition to forecasting broader trends in AMR [62]. Pooling key AMR data and metadata will be essential towards informing such models, as they will fail without large volumes of comparable data. Comprehensive, centralized AMR data and metadata management systems with user-friendly interfaces could facilitate such efforts [63]. Such efforts applied to environmental surveillance of AMR could similarly be a powerful tool towards identifying factors driving AMR globally, including, socioeconomic indicators, and assessing and refining the efficacy of local policies (such as eliminating or reducing the use of certain antibiotics) towards reducing AMR burden [64••].

It is critical to recognize that global health stands to broadly and comprehensively gain from the establishment of environmental monitoring infrastructure proposed herein. In addition to informing strategies to stem the spread of AMR, such networks can easily be expanded to address various bacterial, viral, fungal, and parasitic pathogens. In concert with the microbial monitoring described herein, direct measurements of antibiotics and other antimicrobials could also help to fill critical reporting gaps with respect to antimicrobial use and consumption [65]. Recent reports of illegal drugs in sewage [66] have further demonstrated the potential broad information that can be gained to broadly address greater public health ailments. Now that the general public has grown accustomed to freely available public health dashboards, for example, following day-by-day COVID epidemiological curves, we may have reached a place in society where the value of such transparent information is broadly accepted and appropriately acted upon.

Conclusions

The COVID-19 pandemic provides a warning of the dire consequences of an uncontrolled global public health threat, but also an exemplar and path forward with respect to key infrastructure and data sharing that has been emplaced that can be adapted towards coordinated global surveillance of environmental AMR. The need for environmental surveillance as a critical dimension of One Health policy is rapidly being recognized and there is good synergy with several World Health Organization initiatives and NAPs. Stemming the spread of AMR is more challenging in many ways, given that it is continuously evolving and involves a multitude of strains of bacteria and other microbes and corresponding resistance determinants. Culture-based and molecular-based methods for tracking AMR have evolved substantially over the past decade and can be standardized in a way to ensure comparability across studies and validity for inclusion in models to identify drivers of AMR and to predict overall trends. Such standardized methods can readily be employed in a tiered fashion, with centralized sample archives and analysis, to ensure accessibility for LMICs. Data sharing and public-facing dashboards can help make the data broadly accessible and informative to clinicians, veterinarians, policy-makers, and regulators to spur comprehensive advancement of policy and practice that helps effectively stem the spread of AMR.

Author contribution

Amy Pruden: Conceptualization, Writing-Original draft preparation, Supervision, Funding Acquisition. **Peter J. Vikesland:** Conceptualization, Writing-Reviewing and Editing, Funding Acquisition. **Benjamin C. Davis**: Writing-Reviewing and Editing, Data Analysis, Literature Review, Figure Preparation. **Ana Maria de Roda Husman:** Conceptualization, Writing-Reviewing and Editing.

Conflict of interest statement

Amy Pruden is currently serving in consultation to the United Nations Environment Program (UNEP) regarding development of a report offering guidance with respect to international cooperation in the management of environmental dimensions of antibiotic resistance. Release of this report is anticipated in 2022. This is a purely volunteer effort with no financial compensation. There is no other potential conflict of interest among the authors, including financial conflict.

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- • of special interest
- •• of outstanding interest

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