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Applying systems thinking to analytical system development for managing the antimicrobial resistance crisis

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Antimicrobial resistance (AMR) is an issue with foundations in clinical and agricultural sectors that has become a cross-sectoral and global human, animal, and environmental health crisis. Advances in detection technology and data sharing will allow for quick diagnostics and treatment, real-time monitoring and analysis to prevent AMR exposure, and multi-variate spatiotemporal trend analysis on the dynamic issue that is AMR to make informed interventions and policy. AMR samples are widely diverse in their matrices including blood, urine, wastewater, environmental waters, and soils. Samples can contain many interferents, particularly other contaminants like metals, microplastics, and organic pollutants, that can influence the apparent AMR. Thus, there is a need to drive innovation in cost-effective, rapid, and portable detection technologies and standardized testing methodologies for monitoring, understanding, and managing the complex AMR crisis.

Introduction

Antimicrobial resistance (AMR) is a contaminant of emerging concern (CEC) with projections for growing impacts on environmental and human health.[1–3] This has generated global calls for international cooperation to formulate a global response to AMR. We echo this call for international, multi-disciplinary, cross-sectoral, cooperative action, including comprehensive surveillance, with recognition of the complexity and interconnectedness of AMR across sectors and geographic regions.[1–3]

Antibiotic-resistant bacteria (ARB) and AMR have been an alarming issue in clinical practices for as long as antimicrobials have been synthesized en masse for clinical use beginning with the isolation of penicillin by Sir Alexander Fleming.[4–7] The race to find more antimicrobials is, however, only one branch to approach this developing crisis and alone is insufficient in response to AMR. A single antimicrobial cannot effectively target all microbes due to the differences in mechanisms of action due to their differences in biology and, as we have continuously observed, resistance will continue to arise to new agents rendering them ineffective.[7–11]

A 2017 World Bank report included economic simulations showing that a low AMR impact scenario could cause a loss of over $1 trillion USD annually in global gross domestic product by 2030, and a high-impact scenario with losses or over $3.4 trillion USD annually.[12] Estimates showed, that by 2050, AMR could cause up to 10 million deaths and result in $1 trillion USD in additional healthcare costs.[12] The Council of Canadian Academies reported that AMR caused an estimated 14 000 deaths, and $1.4 billion in expenses to the Canadian healthcare system in 2018.[9] Another estimate from 2022 suggests that a nearly 5 million deaths globally in 2019 could be associated with AMR, putting AMR in third place for the global burden of disease causing death, behind only stroke and ischaemic heart disease.[13] Many experts and governing bodies agree that the largest health and economic impacts of AMR will be on communities living in poverty and that the poorer countries suffer the most from the global AMR crisis.[9,12,14–16]

Establishing shared knowledge and terminology

The authors of this review work in multidisciplinary and interdisciplinary fields and understand that differing terminology and jargon create barriers in communication and understanding. For clarity, this section creates a shared vocabulary by defining key terms, with disciplinary context, to facilitate the discussion about AMR across the various disciplines and sectors. These definitions create a base for a shared understanding and to inform decision-making, particularly within bioanalytical experimental considerations. Firstly, classifying AMR as a CEC may be odd to our analytical community given that AMR is a developing phenomenon rather than a discrete

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entity. Additionally, the term contaminant in analytical sciences would refer to a specific analyte or set of analytes. In this case, specific analytes such as ARB and antibiotic resistance genes (ARGs) would be what are measured to characterize and assess the magnitude of AMR. Often, AMR is referenced to what is being detected.[17–20] As antimicrobial agents (AMAs), AMR microbes (bacteria, fungi, viruses, parasites), and ARGs increasingly entered and disseminated through the environment at the global scale, AMR has become closely associated with pollution.[1,16,21–23] AMR itself is a measurable major health concern and is used as the conventional umbrella term for the total account of AMR-related contaminants that needs to be monitored, managing the spread of, and mitigating the changing impacts of. The term "emerging" with respect to AMR as a CEC refers to the recent, emergence as a threat and the expansive transport and dissemination of AMR resulting in a changing problem that has a lack of effective implemented management strategies due to lack of understanding and lack of effective detection ability.[3,9,13,16] While AMR has existed for a long time, the increasing resistance rates, severity of infection, prevalence in multi-drug resistances, and the consequential health outcomes show that AMR and the AMR crisis continue to rapidly evolve as a critical global health and scientific concern.

Systems thinking is an approach to understanding problems by focusing on how multiple units or sub-systems interact through structured relationships (governed by societies' policies) rather than considering each component in isolation.[24–28] Studying AMR through systems thinking recognizes that systemic socio-ecological structures affect the emergence, dissemination, and impact of AMR, and ultimately, the effectiveness of our mitigation responses.[25–28] Acknowledging the AMR crisis as a complex, dynamic, and adaptive system is simultaneously hopeful and sobering, it shows that we can affect change but also that our (in)actions have multiple implications in this interconnected system. A systems thinking approach results in multiple data sources from different sectors being integrated and analysed holistically to inform policy, prevention, and intervention. By breaking down the AMR crisis into smaller sub-systems and micro-level interactions and how these influence the broader system network, the significance of potential AMR drivers, transport and dissemination pathways can be further clarified and understood.

Furthermore, some historical and microbiological background is provided to create a similar knowledge base to include readers from various backgrounds and familiarity with AMR. This empowers all readers to fully engage in the analysis and discussion of AMR in the micro to macro context, through the lenses of bioanalytical chemistry and systems-thinking to foster an appreciation for the complexity of the AMR crisis.

This review is structured to guide readers from fundamental interactions at the microscale between AMR to systems-level considerations to improve analytical methodologies for effective and sustainable responses to the global AMR crisis. Firstly, we situate the discussion to be engaged with the One Health framework. Beginning the main discussion, localized interactions between AMR and co-occurring contaminants are examined to determine the influences co-contaminants have on AMR and on sample analysis. The discussion then expands to transport pathways through which AMR and co-contaminants move within and across interconnected One Health sectors (human, environmental, and animal and plant health), highlighting key AMR environments, as shown in Fig. 1. Readers are encouraged to refer to Fig. 1 to ground the discussions of AMR at the microscale through to sector-specific environmental analyses, as each contribute to the larger, interconnected, and complex AMR crisis. The review then connects these AMR analyses to the need for coordinated monitoring strategies, emphasizing how standardized methodologies, harmonized reporting, and cross-sector communication are needed to establish a larger integrated analytical framework and systematic interoperable databases. This enables further bioinformatics and machine learning analytics for identifying localized and global AMR trends. Finally, the paper proposes recommendations towards a comprehensive approach centred on innovative sensing technologies, robust diagnostics, and global collaboration to improve AMR surveillance, prevention, and treatment infrastructure while supporting evidence-based policy development.

Antimicrobial resistance: emergence, mechanisms, and spread

AMR is a phenomenon broadly described as the mechanisms by which evolving microorganisms survive the antimicrobial agents (AMAs) intended to inhibit them, threatening public health.[8,9,16,29–31] However, AMR is not a new phenomenon as microbes have been evolving in community and competition with each other for millions of years.[4,8,10,23,31–33] For example, gram-negative bacteria with both an inner and outer membrane have better resistance to environmental pressures (like metals) and to many antibiotics that are effective on gram-positive bacteria which only have a single membrane.[10,11,34,35] Many AMAs, such as antibiotics, are natural products isolated from microbes that produce them to inhibit competitor microbes.[4,10,22,23,30,33,36] These original AMR mechanisms by which microbes have evolved over millions of years of competition before anthropogenic interference are commonly referred to as "intrinsic resistance". However, new interactions with new abundances of AMAs in the environment have applied selective pressures for AMR.

Humans have used plants and fungi to treat disease for millennia. The discovery of penicillin by Sir Alexander Fleming in 1928 was a result of his curiosity about a repeated observation in his Petri plates that a mould, *Penicillium notatum*, inhibited the growth of *Staphylococcus aureus* culture.[4] Penicillin was successfully mass-produced to treat bacterial infections transforming modern medicine. However, resistance to penicillin in hospitals became apparent by the 1940s and continued to spread to bacteria strains in the larger community by the 1970s.[5,6] The discovery, isolation, mass-production, and (mis) use of subsequent antibiotics and other antimicrobials ultimately resulted in "acquired resistance" to each new drug. Microbes acquire resistance through genetic mutations or horizontal gene transfer (HGT) to combat antimicrobials, and

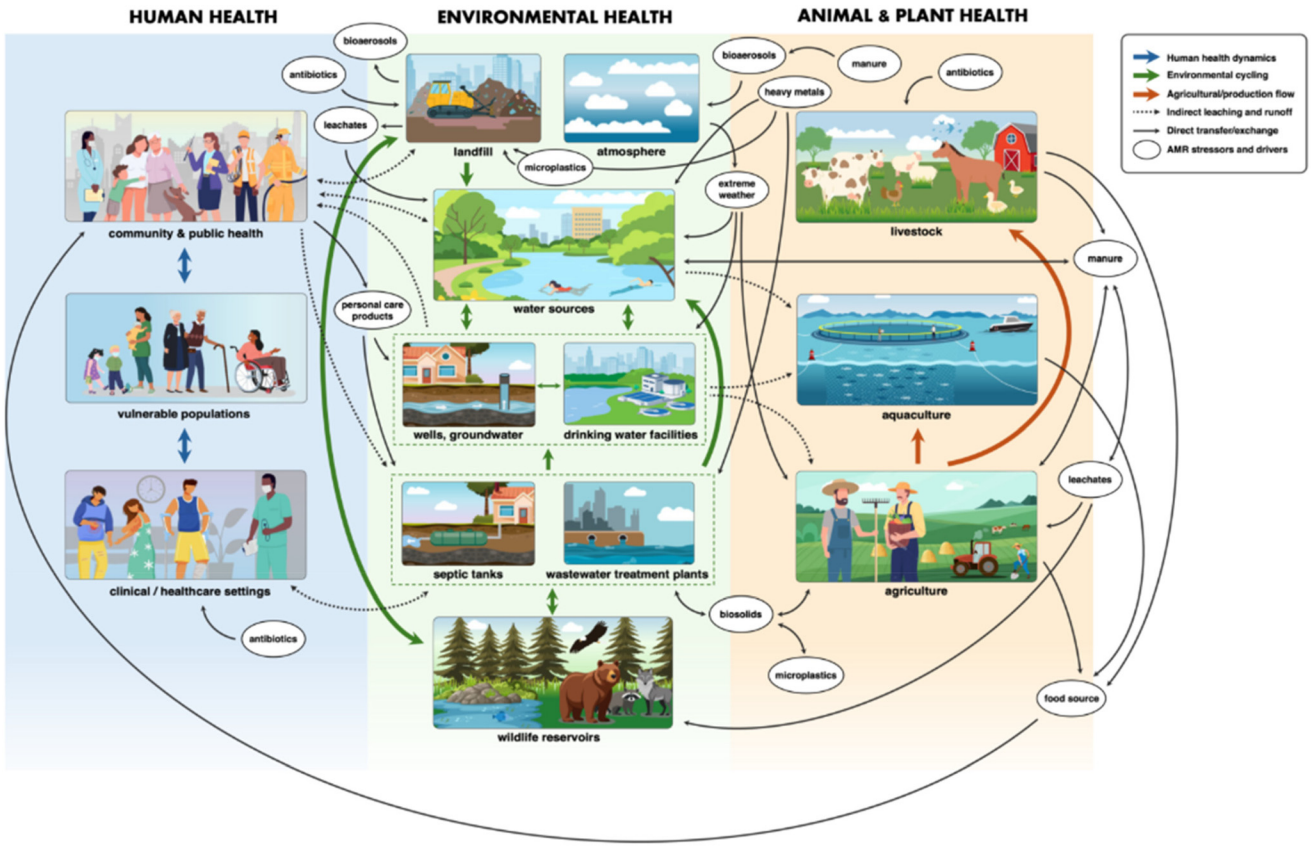

Fig. 1 Schematic representation of antimicrobial resistance (AMR) dissemination across sectors of the One Health framework. Human, agricultural, and environmental sectors are connected through multiple pathways, including water, food, waste, and atmospheric processes. These interlinked routes showcase how AMR circulates across systems and affects all interconnected domains. Water is the primary transport pathway for AMR. Solid and thick arrows represent transport of AMR within sectors, dotted and thinner arrows represent transport of AMR *via* leaching and/or runoff, and solid thinner arrows connect AMR stressors and drivers to key environmental settings within and between sectors.

then survive and produce progeny with such advantageous genetic mutations.[7,10,32,37–44] Generally, mechanisms of AMR can be sorted into three categories according to the biochemical pathway associated with the resistance:[10,11,34,35,45–49]

I. Producing enzymes that modify and inactivate the antibiotic molecule by altering it (such as by acetylation) or by destroying it (such as hydrolyzing the β-lactam ring of β-lactam antibiotics).

II. Changing the target site of the antibiotic.

III. Preventing the antimicrobial from reaching the target by inhibiting uptake or actively effluxing the antibiotic compound.

Bacteria can share DNA, including ARGs, with each other through HGT. There are three mechanisms of HGT:[21,31,33,37,40–44,50–60]

I. Transformation – a bacterium releases DNA into their local environment and is taken up randomly by nearby bacteria.

II. Conjugation – bacteria can transfer genes to other bacteria *via* direct contact.

III. Transduction – genes are transferred to bacteria *via* bacteriophages.

These mechanisms can be further mediated by mobile genetic elements (MGEs), such as plasmids, that can obtain bacterial or other microbial DNA and move them to other cells. Sharing of ARGs becomes more efficient when bacteria are physically bound in proximity, such as in biofilms or attached to microplastics in the environment.[37,43,44,61–65] Bacteria have co-evolved in diverse communities with other microbes (other bacteria, fungi, viruses, *etc*). While it is rare and the mechanisms are not fully understood, HGT has been observed between bacteria and fungi, amongst fungi, and with algae too.[40,52]

Mechanisms of resistance and of HGT are important considerations for detection and surveillance of AMR, including new method development. Detection methods can distinguish ARB from other bacteria by targeting biomarkers and other molecules linked with resistance mechanisms or genes. HGT illustrates the importance of including resistance gene detection in samples where clinically associated ARB may be absent. Viral DNA and RNA, along with ARGs, have also been observed to be transported by bacteriophages viruses that infect bacteria.[31,37,52,53,56–59] This dynamic exchange of genetic information and ARGs specifically leads to increasing

amounts of ARB and AMR microbes found in the environment.

Why it is important

Historically, AMR has been regarded as just a clinical and public health issue. Mechanisms of AMR and ARGs continue to be studied to inform clinicians and develop novel pharmaceutical treatments of infections. It is important to study AMR in microbes to understand AMR mechanisms and to develop therapeutics, detection, and diagnostics technologies. Research has shown that AMR has been a growing problem in sectors such as agriculture,[8,29,66–73] clinical,[8,37,54,74–85] and waste management[42,62,80,84–96] and ultimately, these sectors are all sources for AMR and ARGs in the environment. AMR cannot be meaningfully addressed without using a holistic approach and studying cross-sector dynamics. These sectors are interconnected, particularly with water being a significant transport mechanism of AMR allowing movement across all sectors at both the local and international scale (Fig. 1). Due to anthropogenic activities and globalization, the impact of AMR and ARGs could be far from the source.

Combatting AMR effectively will require interdisciplinary international efforts to fully understand AMR patterns, trends, and transport. Regulators cannot create effective policies or justify resources to implement surveillance programs around AMR because there are too many unknowns due to lack of data, yet institutional support and funding is not sufficient to promote research and development. In addition, research funding differs across microbial life domains resulting in large gaps of knowledge and delayed response. For example, less than 1.5% of all infectious disease research funding is awarded to fungal infections, resulting in treatment guidelines informed by limited evidence according to a 2022 World Health Organization (WHO) report.[97]

Cooperation and communication across sectors and globally can greatly improve data quality, standardize AMR surveillance schedules and techniques, create vast data sets for bioinformatics or machine learning techniques, calibrate AMR, and ultimately inform policies surrounding antimicrobial usage, clinical treatments, and AMR surveillance and management. To understand AMR trends of release, spread, exposure, and infection, we must be able to monitor AMR. To be able to monitor AMR, we require innovative development of robust and fast detection and diagnostics technologies to collect vast amounts of data. The data collection also introduces a factor where standardized methodology, and reporting units will be necessary to effectively compare and communicate findings so that professionals across all sectors globally are on the same page.

One Health framework

The One Health framework, while lacking a single, universally accepted definition, is generally understood as an integrated approach that aims to optimize the health of humans, animals, and the environment through coordinated, cross-sector collaboration. As noted by the One Health High-Level Expert Panel (OHHLEP), the term has been interpreted in different ways across institutions and the literature, which led to the development of a shared working definition to support consistent understanding and application among global partners (WHO, FAO, UNEP, WOAH).[98] Even still, different definitions arise from lack of culturally appropriate translations of the term.[99] The framework emerged from the growing recognition that health outcomes are shaped by the interactions between people, animals, and ecosystems.[100] While its roots can be traced back to the 19th-century work of Rudolf Virchow, who emphasized that there should be no dividing line between human and animal medicine, the framework began to take shape in its modern form in the early 2000s.[101–103] One Health gained prominence during the 2000s, particularly in response to the SARS outbreak and the spread of highly pathogenic avian influenza.[101–104] These events led to increased cross-sectoral collaboration and the development of strategic frameworks to prevent and respond to zoonotic threats.[101–104]

Interestingly, One Health does not recognize nor credit the already existing deeply embodied knowledges, respect, and praxis of reciprocity and relations to the land, wherein the understanding that there are not divisions between human and animal individuals, communities, ecosystems, the environment, and mother earth has holistically served Indigenous peoples' emotional, intellectual, physical, and spiritual health globally since time immemorial[105–110,112] These values and knowledges are reflected in traditional teaching such as the seven generations teaching of many Indigenous nations here on Turtle Island,[107,111] and in frameworks that were co-created by Indigenous scientists and knowledge keepers such as traditional ecological knowledge,[100,107] two-eyed seeing (in the Canadian context),[113,114] and both-ways (or two-ways, in the Australian context).[114] Centring and ethically collaborating with Indigenous knowledge keepers with intention and commitment to reciprocate knowledge and solutions can strengthen relationships and enrich Western science frameworks like One Health. One example where Indigenous knowledge keepers' local ecological knowledge can improve analytical methodologies in the context of monitoring and managing the AMR crisis is in the selection of AMR monitoring sites and in changes to AMR sampling frequency and/or sample types (water, soil, *etc.*) due to factors such as animal activities. Other highly desired and aligned to One Health outcomes of including Indigenous ways of knowing into One Health would be prioritizing locally practical solutions and increased engagement and commitment by local community members[115] in healing major One Health sectors (human, animal, environment) through active participation in scientific exploration and applying solutions. Participation in shared creation and management of knowledge also builds trust and brings in new voices when applying knowledge, particularly important on an application and policy level for tackling AMR. Indigenous communities rightfully have distrust in western health systems which can be alleviated by allowing participation and weaving newly created knowledge with existing Indigenous knowledge frameworks with proper recognition.

AMR has traditionally been treated as a clinical problem, with most efforts focused on the misuse and overuse of antibiotics in healthcare settings.[8,82] However, this limited view has shifted as growing evidence highlights how resistant pathogens and genes move between humans, animals, and the environment.[1,16,22,116] Wastewater discharge, agricultural antibiotic use, direct animal-human interactions, and global trade have shown that AMR is not only a clinical concern but a systemic one.[1,3,101,117] Water, considered part of the environmental domain within the One Health framework, plays a critical role in linking human, animal, and ecosystem health. It acts as a carrier for nutrients, pollutants, and microscopic particles, and provides a microbial habitat that enables the dissemination of ARGs and AMR microbes.[7,39,61,62,92,118–121] Framing AMR through a One Health lens allows for more coordinated and sustainable solutions. It shifts focus from isolated, clinical interventions to broader strategies that address upstream drivers such as pharmaceutical pollution, agricultural practices, and weak AMR surveillance systems.[98] The widespread influence and increasingly alarming consequences of AMR is not limited to health implications but also to economic stability and social equity at the global scale. Thus, AMR can also be viewed in the context of the United Nations Sustainable Development Goals (SDGs) highlighting that AMR is both a scientific and societal challenge.

Detection and monitoring AMR in these various contexts requires an informed consideration of AMR interaction with smaller and more manageable components of these larger systems. Understanding that localized interactions of AMR to other individual contaminants will affect their emergence, AMR mechanisms, and dissemination. This can further be expanded to understand how cumulative effects of multiple contaminant interactions could influence AMR within larger environmental and sectoral contexts that have their own unique influences and sample types. Ultimately, having a better understanding of the effects on AMR of contaminants, other interferents, and matrices that make up the components of a 'real' sample will help create sensing methodologies and technologies with more targeted mechanisms.

AMR community interactions with other contaminants

Many studies on AMR are completed either in laboratories or with isolated microbes that were culturable from environmental samples. The understanding of the biochemical mechanisms of AMR in isolated microbes in clean conditions provides a foundation for researching how AMR interacts with other contaminants and other microbes in the environment. Development of detection methods must consider aspects of "real world" samples because they may affect multiple aspects of detection from extraction and availability of biomolecule targets to presence of interfering components in the sample matrix. Table 1 at the end of the section summarizes the interactions of AMR with the following contaminants discussed.

Biofilms

Biofilms are surface-attached microbial communities that are embedded in a matrix of extracellular polymeric substances (EPS) that are excreted from the microbes.[122,123] Most natural environmental microbial life and communities exist in/as biofilms as well as in some engineered biotechnology environments. Biofilms are found everywhere from within both natural and built aquatic systems (*e.g.* rocks in a river, drinking water pipes) or to touch surfaces (*e.g.* countertops), and into the human body (*e.g.* dental plaque). Biofilms can be very diverse and spatially heterogeneous in microbial, macromolecule, and abiotic composition.[61,63,64,122,123] The structure and composition of the biofilm are dependent on the environment that the biofilm is in and the microbial composition which may contain single or multiple microbial species. The EPS matrix is also variable in composition of macromolecules as well as environmental organic and inorganic materials like environmental DNA (eDNA), metals, and/or microplastics that can be incorporated into the biofilm when they adsorb onto them.[43,44,61–64,120,122–126] The spatial composition of biofilms can also create various microenvironments within biofilms, which can alter the microbial community within that microenvironment. Smaller pieces of mature biofilms can shed off from forces of water, travel potentially large distances, and establish a biofilm elsewhere.[61,122,123]

Table 1 Summary of contaminant Interactions with AMR

Contaminant	Influence on AMR	Ref.[a]
Biofilms	Facilitates HGT, AMR reservoir, reservoir for contaminants like metals and microplastics	43, 44, 61–63, 65, 120, 122 and 123
Metals	Co-selective pressure, binds DNA, damages DNA, increases HGT	39, 61, 72, 124, 128, 130, 194 and 195
Microplastics	Adsorbs DNA, damages DNA, AMR reservoir, biofilms reservoir, transports biofilms, increases HGT, increases expression of efflux pumps	61, 63, 138, 145, 146, 148, 153 and 155
PCPs	Co-selective pressures, damages DNA, increases HGT, increases expression of efflux pumps	49, 50, 159, 161, 163, 165 and 170
Pesticides, herbicides, VOCs, PAHs, and other organic contaminants	Co-selective pressures, binds DNA, damages DNA, increases HGT, increases expression of efflux pumps	175, 177, 180, 184, 186–188, 191 and 196

[a] Non-exhaustive list.

Biofilms are microenvironments that can facilitate microbial community dynamics such as nutrient exchange, cell signalling (*i.e.*, quorum sensing), cooperation and competition, and HGT. All mechanisms of HGT can be found in biofilms due to their retention of DNA and MGEs in the EPS, having cells in close proximity, general environmental stability resulting in prolonged survival, and interactions such as genetic exchange, and proliferation of diverse microbes. Genes from the environment, including ARGs, can also adsorb to and be absorbed into biofilms.[43,44,62,64,65,122–124] Biofilms can thus be hotspots for ARGs and can result in multi-drug-resistant microbial communities.

Metals

AMR co-occurs with metals and other heavy elements in our environment, particularly in the soils and waterways. Currently, the anthropogenic levels of metals in these environments are several orders of magnitude greater than the levels of antibiotics, and unlike antibiotics, metals are not subject to degradation.[127–129] Concerningly, even when there are no AMAs present, certain metals (Pb, Cu, Cr, Zn, *etc.*) can maintain or increase bacterial resistance by exerting long-term selection pressure.[72] For example, Zhang *et al.* (2018) found that sub-inhibitory levels of these metal ions (similar to those found in polluted environments) can accelerate the HGT of ARGs, as the presence of metal ions significantly up regulated the expression of *ompA* and *ompC* genes, which are associated with transcription.[130] Thus, it could be useful to monitor for these transcription related genes in samples where metals are known to be present.

Not only do metals exert selective pressure for antibiotic resistance, the presence of metals within the sample matrix have been shown to interfere with bacterial detection. A common technique used for AMR detection, PCR, has been shown to face inhibition due to metals at various points during DNA analysis including extraction and PCR amplification, as positively charged metal ions have a high affinity for DNA due to its negatively charged phosphate backbone.[131–135] Sagripanti *et al.* (1991) found that Cu specifically, has high binding affinity for the DNA bases.[136] The level at which metals hinder PCR analysis is largely dependent on the concentration and type of metals involved.[132] For example, Wedrychowski *et al.* (1986) found that Hg, Cu, Pb, and Al produce extensive cross-links between DNA and proteins, which can inhibit PCR by blocking access to the DNA template.[133] In another study, Kuffel *et al.* (2020) found that Zn, Sn, Fe(II) and Cu ions have the greatest potential to interfere with DNA amplification with 50-+% PCR inhibition at concentrations of 0.26, 0.31, 0.59 and 0.77 mM respectively.[132]

Nano- and microplastics

Nano- and microplastics are ubiquitous environmental pollutants that can interact with microbes such that they can facilitate the development and spread of AMR, such as *via* growth of biofilms on their surfaces. The high surface areas of microplastics are ideal for biofilm formation and biofilms are known to facilitate HGT including ARGs.[10,61,63,122,137–145] These microbial communities, potentially reservoirs for AMR, are transported wherever the microplastics are transported. Water can transport the biofilm-carrying microplastics large distances and through various ecosystems effectively spreading AMR.[7,16,22,137,139,140,143,144,146,147] This can be of great concern for microplastics passing through environments such as wastewater treatment plants (WWTPs) which are already hotspots for AMR.

Microplastics also allow the transport of biofilms into and up the food web through ingestion. Nanoplastics have also been observed to enter microbes through the lipid bilayer or *via* endocytosis – these will enter the food web if these microbes are ingested.[137,140,144,146–152] Absorption of nanoplastics into cells can influence and change microbial populations because they can create oxidative stress in cells, causing oxidative damage to DNA that can lead to mutations. Consequently, cells undergo SOS response with upregulated expression of core conjugation genes, facilitating HGT including any ARGs.[148–154] Finally, microplastics can adsorb and transport other contaminants such as antibiotics and metals synergistically with DNA and cells, which can create microenvironments of selective pressures on any attached microbes.[61,138,140,143,148,155] These influences of nano- and microplastics on AMR creating ARG-rich biofilms could result in increased ARG detection within environmental samples containing higher amounts of microplastics. While this possible overestimation could be considered as a conservative estimate, it would introduce a bias and may misrepresent the patterns and trends of AMR and ARGs at a monitoring site determined using that inaccurate data.

Association of ARB and ARGs with nano- and microplastics, including in biofilms on plastics, can interfere with the detection of biomarkers and other targets. If the analyte is eDNA, where in an environmental sample may contain various abundances along with metal ions, nano- and microplastics, the eDNA can be damaged or adsorbed, consequently affecting any genomic analyses.[61,152,155] Any environments that are reservoirs for nano- and microplastics must be studied in how the microplastics are transported as well as how they may be potential reservoirs for ARGs and AMR.

Personal care and pharmaceuticals products

Personal care products (PCPs), including cosmetics, soaps, shampoos, lotions, toothpastes, and disinfectants, also play a significant role in the prevalence and spread of AMR. Many PCPs exert selective pressures on microbial communities in the environment, including domestic and commercial environments, largely because they contain AMAs to inhibit microbial growth and extend PCP shelf life.[156–163] Generic use of PCPs results in the component compounds, including AMAs and surfactants, entering the wastewater system. These compounds are not fully removed by the current wastewater treatment processes and are released into the environment. For example, triclosan and triclocarban are AMAs and preservatives found in thousands of PCPs that have shown to persist in the environ-

ment, contribute to AMR, and bioaccumulate in the food web.[156–160,164–169] A 2007 mass balance assessment found that 1.1×10^5 to 4.2×10^5 kg of triclosan are discharged into the environment by WWTPs in the US annually.[166] Triclosan has been found to accumulate in anaerobic digestors, WWTPs sludge, and aquatic ecosystem sediments, and has been observed to influence the microbial communities, promote HGT, cause genetic mutations, and select for resistant microbes.[54,157,159,160,163–165,169]

Other PCP preservatives such as sodium nitrite and sodium benzoate have shown to increase HGT by increasing the expression of *trfAP* and *traJ* genes by up to 17-fold in a concentration-dependent manner.[169] Another major component of PCPs are surfactants, such as benzalkonium chlorides (also classified as a quaternary ammonium compound antiseptic), can select for AMR through multiple mechanisms including mutagenesis, upregulated expression of multidrug efflux pumps, and changing membrane fatty acids to modify cell surface charge.[49,170] Upregulation of different efflux pumps has been a prominent observed resistance mechanism to antiseptics.[49] LaBreck *et al.* (2020) determined that pre-exposure to one antiseptic can increase resistance to another antiseptic suggesting a co-selection for multiple resistances.[49] Other non-antibiotic pharmaceuticals, including the commonly prescribed antiepileptic carbamazepine, significantly promote HGT and thus the dissemination of ARGs.[50,54,163] The commonly prescribed antidepressant fluoxetine has been found to induce AMR in *Escherichia coli* (*E. coli*) to multiple antibiotics *via* genetic mutation and upregulated expression of multidrug efflux pumps.[171] The microbial communities within the WWTPs and in the environment are continuously exposed to these PCPs and the selective pressures they exert, promoting the proliferation of AMR.[80,81,163,172]

Pesticides and volatile organic compounds

Pesticides, including herbicides, fungicides, *etc*, are chemicals used largely in agriculture but also in urban areas to deter, control growth of, or eliminate unwanted pests and plants.[173,174] The concern for environmental pollution of pesticides and herbicides, largely from agricultural runoff, may be more consequential given the unintended effects of promoting AMR.[67,70,173–182] Exposure to these chemicals can increase the abundance of ARGs in soil microbiomes by triggering cell defence mechanisms including upregulation of multidrug efflux pumps and enhanced HGT.[48,67,174,175,178,182,183] The altering of microbes and microbiomes *via* herbicides and pesticides not only alters human pathogens but also in phytopathogens that are directly responsible for the loss of agricultural quality and yield.[173,184,185] Glyphosate, a widely used herbicide against weeds and a main chemical in Roundup, has demonstrated that its use and exposure promotes AMR particularly through the increased mobilization of ARGs and MGEs, upregulated expression of efflux pumps, and/or reduced expression of outer membrane porins.[67,174,175,178] Rangasamy *et al.* (2018) determined that microbes collected from agricultural fields that were resistant to organophosphate pesticides were also resistant to antibiotics because α–β hydrolase enzyme that biodegrades the organophosphate pesticides are capable of degrading antibiotics.[180] These findings suggest that pesticides can exert a co-selection pressure for AMR and pesticide resistance.

Other organic environmental stressors include volatile organic compounds (VOCs), solvents used at industrial scales, and polycyclic aromatic hydrocarbons (PAHs). Complex interactions with these organic pollutants result in differing effects on AMR development. Bacterial tolerance to solvents, like toluene and ethylbenzene, involve efflux pumps which have been shown to simultaneously remove multiple antibiotics, like ampicillin and tetracycline.[186,187] Bacteria able to biodegrade VOCs and PAHs have also been shown to have increased AMR to multiple antibiotics, again through the mechanism of efflux pumps and show increased MGEs.[179,182,188–190] These studies suggest multi-resistances and co-selective pressures for these organic pollutants and AMR.

Environmental soil or water samples can contain pesticides, herbicides, PAHs, and/or VOCs that can affect genomic analyses. Several possible effects on the detection and quantification of ARGs in environmental samples include damaging nucleic acids, inefficient extraction of eDNA/RNA extraction due to adsorption to the soil, the organic interferents being extracted along with eDNA/RNA, inhibiting PCR *via* nucleotide adsorption, and/or inhibition of DNA polymerases.[191–193] These effects can produce false negatives due to the apparent low abundance of ARGs.

Together, these studies illustrate how co-contaminants could interfere with sample analysis or co-select for AMR. Taking these effects into consideration can help better interpret AMR results, consider sample pre-treatment, and/or select target AMR analytes such as ARGs or efflux pumps in contexts where co-contaminants may be highly suspected to be in the sample. Another consideration could be to include multi-analyte analysis that couples chemical and microbiological measurements to interpret AMR results alongside these co-contaminants and co-selective pressures. Including multi-analyte data can further determine if remediation of co-selective AMR drivers would need to be considered during an intervention.

Common possible forms of AMR introduction

Biofilms and microplastics

As discussed in the previous section, biofilms and microplastics each have unique interactions with AMR. Biofilms can be hotspots for ARGs and can result in reservoirs of AMR and ARGs.[10,197] Biofilms also offer a transport pathway for AMR and ARGs as mature biofilms can often split off pieces that can travel *via* water to a different location where the biofilm can continue to form and proliferate.[1,43,44,61,62,64,65,122,123] Biofilms often form on microplastics, easily colonizing the high surface area substrate, in both aquatic and terrestrial

environments.[137,138,140,141,143,144] These microplastics can be transported through various mechanisms, such as with water or in air, ultimately transporting the attached biofilms with them. For example, AMR and ARGs could enter the food chain when fish consume microplastics that have these biofilms on them.[137–140,143,144,146] Ultimately, the transport of microplastics is of great concern with respect to AMR because AMR may go wherever the colonized microplastics go. This could mean transporting and introducing AMR to new ecosystems, new microbial communities, and into the food chain.

Bioaerosols

A phenomenon that is common yet did not receive much public attention prior to the SARS-CoV-2 pandemic is a bioaerosol.[198,199] Bioaerosols can be composed of various living and dead microbes (bacteria, fungi, viruses, archaea, *etc*) including any spores, pollen, or fragments of cells debris.[200,201] Bioaerosols are consistently generated from various natural sources such as from soil surfaces, water bodies, and animals.[200–203] Anthropogenic activities are also a major source of bioaerosols such as in the collection, transport, and landfilling of wastes, wastewater treatment, agricultural practices, and more.[23,68,202,204–209] Weather events such as wind and rain can facilitate the generation and transport of bioaerosols.[206,210] It has been reported that 0.01% of bacteria from soil could aerosolize when a raindrop hits the soil surface, dispersing microbes into the air.[202]

Microbes in bioaerosols can be transported by the wind and can deposit *via* gravity or by precipitation.[201,206,210,211] Depending on climate conditions, bioaerosols can travel large distances through different conditions and the microbial composition and particle composition may change; this also applies to small and/or indoor spaces.[162,206,207,211–213] Studies have shown that dusts of bioaerosols can remain in the atmosphere for a long period of time, being able to compositionally change with already aerosolized microbes resulting in high microbe diversity. A metagenomic study on samples collected during a Nov–Dec 2014 dust event in Seoul, South Korea, where 16S rRNA genes sequenced *via* pyrosequencing revealed that there was high bacterial diversity showing 19 phyla and further characterization found 646 genera present.[214] Another Asian dust event study by Tang *et al.* (2018) showed their samples contained 34 bacterial phyla (further characterized to 243 families) and 3 fungal phyla (further characterized to 149 families).[206] Placing this into an agricultural context, Bai *et al.* (2022) showed that microbes and their ARGs in bioaerosols could be detected 10 km away from animal farms.[68]

Migration and AMR mobility

Organisms at all levels of complexity, including carriers of AMR organisms or ARGs, rarely remain confined to one single space or ecosystem. Local travel and travel across landscapes and borders both in natural and human-mediated processes occur continuously, transporting the AMR organisms and ARGs.[2,183,215–217] Many wild animals have deeply innate migratory patterns tied to their life cycles and survival.[218,219] These animals can act as mobile AMR reservoirs acquiring ARB and AMR from contaminated sites, such as from landfills and WWTPs, and potentially dispersing the AMR over large distances during seasonal migrations. Evidence has shown that gulls foraging in landfills can spread ARB to nearby areas and potentially across continents during their migratory season.[217] One can imagine that geese swimming and feeding in wastewater effluent ponds acquire ARB and subsequently fly and introduce AMR to urban parks and recreational waters. The recent ongoing zoonotic pandemic of SARS-CoV-2 is a great example to show the transmission of disease goes both ways with human disease variants transmitting to animals from white tailed deer in remote hunting grounds that been found with SARS-CoV-2 infections to domestic animal friends to wildlife under human care like in zoos.[220,221] Anthropogenic activities on local and larger international scales also contribute heavily to AMR spread. Local communities can be affected by hospital effluent discharges or due to proximity to landfills. Simultaneously, natural weather processes such as precipitation, wind, and worsening natural disasters also carry AMR microbes and ARGs, especially from agricultural lands/run offs and sewage overflows, further disseminating AMR into both adjacent and faraway environments.[32,64,70,88,141,145,163,176,202,206,210,211,222–226] On a global scale, international travel, trade, and mass migrant or refugee migrations can transport AMR into new areas potentially seeding and diversifying AMR genes.[15,215,224,227–229]

One key common factor is the role that water plays in the transport and mobility of AMR. As water moves and flows around the globe in the forms of the environment, weather, and as human and animal bodies, AMR moves with no regard for borders or boundaries.[7,32,62,66,119,121,202,204,215,216,218,219,230–238] The interconnected pathways of AMR dissemination results in the need for a global collective intervention. No single region can eliminate AMR in isolation as limited efforts will be undermined by the continual reintroduction of AMR through the movement of water and anthropogenic activities. The One Health framework provides a method for a coordinated international response to the complex interconnected systematic issue that is AMR.

Standard and emerging methods of AMR detection and characterization

Methods for AMR detection can be categorized as genotypic, focusing on detecting nucleic acids, and as phenotypic, where conventional culturing and bioassays are performed.[222–227] Genotypic methods give us information about the presence and prevalence of ARGs. Phenotypic methods give us information about what AMR mechanism the microbe is using and what ARGs are expressed.[20,239–243] Currently, the standardized AMR detection methods used in testing are the phenotypic Kirby-Bauer disk diffusion method, broth microdilution, Epsilometer test (Etest), and the genotypic polymerase chain

reaction (PCR) – specifically, quantitative PCR (qPCR), sometimes called real-time PCR, is largely used in AMR detection.[20,239,244–248] However, only some antibiotics and bacterial species have been standardized with the disk diffusion method and the meaning of an inhibition zone for a new antimicrobial or different bacterial species would need to be repeatedly validated.[239,245,246] The disk diffusion method also cannot determine the minimum inhibitory concentration (MIC) of the antibiotic like the broth microdilution method.[239] Both methods are constrained by the growth time of the bacteria, do not determine the mechanism of AMR, and need to start with an isolated culture, making them not suitable for fast, reliable testing. Newer genotypic methods such as PCR are faster and useful for identification of ARGs, but still require sample preparation by highly trained personnel in a sterile lab facility; qPCR is also an option, but these instruments are costly and still constrained by the same PCR factors.[239] Another issue with PCR testing is that the presence of ARGs does not mean that the bacteria are expressing them, and we cannot know if those bacteria are actively ARB. These methods cannot currently be used for rapid, routine testing of ARB nor for on-site monitoring, especially in remote areas. Development of a portable, *in situ* testing device would avoid the need for expensive analytical instruments and highly trained personnel.

Researchers developing new methodologies for AMR detection use at least one of the standardized genotypic or phenotypic methods to compare and validate their methods. However, it is valuable to conduct both genotypic and phenotypic methods to screen for ARGs, identify microbes (such as using 16S rRNA to identify bacterial species), and to confirm the AMR phenotypes and mechanism(s). Abandoning culturing and phenotypic methods too quickly in favour of relying on only genotypic tests will result in gaps of data and making misinformed decisions. The correlation of genotype to phenotype is still an interpretive challenge due to various reasons such as gene expression, gene regulation, and post-translational modifications on proteins. Advances in predicting AMR phenotype from ARGs is appealing, but current machine learning models still require complete genome sequence data and results have high error rates.[249–253] It is important to continue to collect large amounts of genotypic and phenotypic data using current and new methods to be incorporated into open databases to ensure data is aligned and validated as new technologies emerge. Creating these large, validated, open datasets produce better training sets resulting in continued advances in integrating machine learning and artificial intelligence for AMR predictions, diagnostics, and spatiotemporal trend analysis. An overview of standardized AMR detection methods along with new emerging methods and technologies, such as biosensors, that have potential to be robust and reliable for AMR detection are presented in Table 2. In depth reviews on the standard and recent advancements in detection methods at various technology readiness levels are abundantly available elsewhere and within the referenced literature.[17–21,239,254–256]

Dedicated validation and standardization efforts, with various sample types, must be pushed on exciting emerging portable methods to advance AMR detection and surveillance capability and capacity. Achieving validated, reliable, robust, and portable methods has become urgent to implement effective AMR monitoring and surveillance. When validated, easy-to-use, portable AMR testing technology is developed, citizen science becomes another approach to educate and include the public which can encourage AMR stewardship.[257–259] When looking at AMR research in different sectors, the analytical methods used to detect and study AMR are the same even though the sample types are very different, requiring different sample collection methodology and, sample preparations, and may be limited to certain techniques. New innovations in technologies must consider the interconnected nature of the AMR crisis across sectors and the ability of new methodologies to reliably analyse AMR across various complex sample matrices.

Overview of AMR detection in diverse samples to support a systems approach

AMR manifests across a variety of biological and environmental systems, each shaped by distinct pressures, microbial communities, and patterns of antimicrobial use. These interconnected domains form key hotspots for the development, persistence, and spread of resistance.[94] Due to the diversity in sample sources – ranging from human and animal health to natural and engineered environments – the underlying complexity and connectivity of AMR demands an integrated approach to analysis to support a systems approach to managing AMR. Interpreting this complexity requires working across disciplines and making sense of large, heterogenous datasets.[95] While many of the analytical tools used to detect and study AMR, such as culture-based methods, molecular assays, and metagenomics, are consistent across fields, their application is highly dependent on the sample type, requiring different preparation protocols.[96] This intersection of shared methodologies and domain-specific challenges underscores the importance of coordinated efforts in AMR research and surveillance.

Clinical

Clinical settings act as intense source points in the broader landscape of AMR, placed within a web of interactions that spans human, animal and environmental systems.[31] The routine use and overuse of antibiotics in hospitals, combined with factors like invasive procedures and patient susceptibility, creates conditions where resistance can emerge rapidly and spread efficiently.[29] The rise and spread of resistant bacteria in hospitals is not confined to any one region or health system, it poses a transnational threat with consequences that cut across social and economic factors.[306] Within these environments,

Table 2 Current and emerging AMR detection methods

Technique name	Description	Sample types and processing	Current application maturity and limitations	Time needed[a]	Example references[b]
Genotypic methods					
Conventional polymerase chain reaction (PCR)	Amplifies specific DNA sequences within a sample using primers for a target gene including ARGs.	Clinical isolates, environmental samples (soil, water, air, sediment); DNA extraction, isothermal amplification	Widely used, established public health use; pre-processing samples, targeted nucleic acid sequences only	2–4 hours	59 and 260
Real-time PCR (qPCR)	Real-time detections and quantification of target genes.	Clinical isolates, clinical samples (blood, urine); DNA/RNA extraction, isothermal amplification	Widely used, established public health use; pre-processing samples, targeted nucleic acid sequences only	1–4 hours	213 and 261–263
Multiplex PCR	Amplification of multiple target genes simultaneously in a single reaction.	Clinical samples (blood, urine); DNA extraction, multiple primer optimization, isothermal amplification	Emerging commercial products for clinical use, AMR research; pre-processing samples, targeted nucleic acid sequences only	2–4 hours	248, 264 and 265
Digital PCR (dPCR)	Absolute quantifications of nucleic acids by partitioning samples to detect and quantify target genes with high sensitivity.	Clinical isolates, clinical samples (blood); DNA extraction, isothermal amplification, partitioning	Used in AMR research, limited but growing clinical use; cost per test, pre-processing samples, targeted nucleic acid sequences only	2–4 hours	266–268
Reverse transcriptase (RT-PCR)	Transcribes RNA into cDNA before amplification; can identify reproducing cells with high sensitivity, making it useful for detecting live ARB.	Clinical samples; RNA extraction, reverse transcription	Advanced research studying expression of mechanisms, sometimes used for diagnostics; pre-processing samples, targeted nucleic acid sequences only	3–5 hours	269 and 270
Whole genome sequencing (WGS)	WGS provides comprehensive genomic information and can potentially detect all AMR-encoding genes in a single assay	Bacterial isolates; library preparation, DNA extraction	Established in public health use and reference labs, emerging clinical use; pre-processing samples, cost, bioinformatics expertise and infrastructure	24–48 hours	55, 75 and 241
Short read sequencing	Technologies like Illumina generate short DNA fragments that can be assembled or directly analysed to predict resistance.	Clinical isolates, environmental samples (soil, water, sediment); library preparation, DNA extraction	AMR research and in labs for AMR profiling, uncommon diagnostics use; pre-processing samples, library preparation, more complex data analysis and interpretation	12–24 hours	271–273
Long read sequencing	Platforms like Oxford nanopore technologies can generate longer reads, allowing for better assembly of complex genomic regions.	Clinical isolates; library preparation, DNA extraction	AMR research, emerging real-time uses; pre-processing samples, library preparation, more complex data analysis and interpretation	8–24 hours	272–274
Hybrid sequencing	Combining short and long-read technologies can provide more accurate and complete genomic information for AMR prediction.	Clinical isolates; library preparation (long and short), DNA extraction	AMR research and reference labs; cost, pre-processing samples, library preparation, more complex data analysis and interpretation	24–72 hours	273, 275 and 276
Amplicon sequencing	Specific PCR-amplified genes or regions of interest, the amplicons are sequenced to detect mutations, copy number variations, *etc.*	Clinical isolates; library preparation, DNA extraction, isothermal amplification	AMR research, emerging public health use; cost, pre-processing samples, library preparation, targeted nucleic acid sequences only, more complex data analysis and interpretation	4–8 hours	51 and 277
Metagenomic sequencing	Detection of AMR genes in complex microbial communities without the need for culturing.	Environmental samples (soil, water, air, sediment), wastewater, wastes; library preparation, DNA/RNA extraction	AMR research, growing public health use; library preparation, cost, bioinformatics expertise and infrastructure, more complex data analysis and interpretation	8–24 hours	82, 90 and 205
RNA sequencing (RNA-Seq)	Provides information on gene expression levels and transcriptional responses of microbes to AMAs, identifying active AMR mechanisms.	Clinical isolates; library preparation, RNA extraction	AMR research; cost, more complex data analysis and interpretation	12–24 hours	50, 277 and 278

Table 2 *(Contd.)*

Technique name	Description	Sample types and processing	Current application maturity and limitations	Time needed[a]	Example references[b]
Pyrosequencing	A real-time sequencing method where a pyrophosphate is released and starts a cascade of enzymatic reactions when the tagged nucleotide is added and generates a light signal.	Clinical isolates; DNA extraction, DNA sequencing	Could be used but largely replaced by newer technologies; shorter read length, limited materials due to disappearing use	4–8 hours	214, 279 and 280
CRISPR-Cas13	Binds to target RNA and uses collateral cleavage activity to generate and amplify signals.	Clinical isolates, bacterial isolates, clinical samples (blood, urine); DNA extraction, library preparation, isothermal amplification, CRISPR reaction	AMR research, successes in proof-of-concept research; pre-processing samples including amplification, no standardized, routine methodology yet	45 mins–2 hours	281–283
Machine-learning enhanced sequencing	Integrating AI to WGS or multiplexed sequencing can uncover new determinants of AMR or predict MICs of ARB based on their AMR profiles. Must be careful of risks of bias in the algorithms.	Clinical isolates, clinical samples (blood); DNA extraction, DNA sequencing	AMR research and proof-of-concept; requires large, high-quality datasets, lack of regulations and standardized methodology	<1 hour	251, 284 and 285
Electrochemical biosensors	A biorecognition element binding a target analyte creates an electrochemical signal. Label-free systems. Can be integrated with microfluidic devices; Ex. An electrode modified with the complimentary DNA probe of an ARG of interest, the electrochemical signal will change with hybridization of the ARG.	Clinical isolates, bacterial isolates, clinical samples (urine, blood), environmental (water); cell lysis, direct electrochemical measurement of nucleic acids	Advanced research with multiple proof-of-concepts, some real-sample success, some prototypes; no standardized, routine methodology yet, detection robustness challenges	30 mins–2 hours	286–288
Optical biosensors	Fluorometric or colourimetric biosensors, can be integrated with smartphone cameras; surface plasmon resonance (SPR) detects real-time changes in refractive index upon target analyte binding, typically gene hybridizations.	Clinical isolates, bacterial isolates, clinical samples; filtration, DNA extraction	Advanced research with multiple proof-of-concepts, some real-sample success, some prototypes; no standardized, routine methodology yet, limited miniaturization due to optical components		289–291
Phenotypic methods					
Kirby–Bauer disk diffusion	Bacterial susceptibility to antibiotics and AMAs by observing and measuring growth inhibition zones around antibiotic disks.	Bacterial isolates; inoculation and culturing plates	Long-established gold standard, routine, reference method; requires isolates, long incubation times	16–24 hours	236 and 246
Epsilometer test (Etest)	Follows the principles of the Kirby–Bauer except a gradient strip of AMA is used to determine the MIC for a bacterial isolate.	Bacterial isolates; inoculation and culturing plates	Widely used; requires isolates, long incubation times, limited validated AMAs	16–24 hours	78 and 247
Broth microdilution	Determines MIC for antimicrobials by assessing inhibition of bacterial growth in liquid medium with serially diluted antimicrobials.	Bacterial isolates; inoculation and culturing of nutrient broth	Gold standard, routine, reference method; requires isolates, long incubation times	16–24 hours	241, 292 and 293
Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) Mass spectrometry	Rapid bacterial identification and AMR detection through protein mass spectra analysis.	Bacterial isolates; cell lysis, protein extraction, matrix application	Established, routine method in some clinical labs and hospitals for identifying microbial species, advanced research for AMR mechanism profiling; specialized equipment, cost, pre-processing samples, no standardized AMR mechanism identification workflows yet	2–4 hours	294–296
Electrochemical biosensors	Electrochemical detection of AMR enzymes (β-lactamases, carbapenemases) and resistance proteins. Can be integrated with microfluidic devices and smartphone devices.	Clinical samples (blood, urine); bacterial isolates, environmental samples (water); direct electrochemical measurement	Advanced research with multiple proof-of-concepts, some real-sample success; fouling by non-specific interactions in real samples and complex matrices, no standardized, routine methodology yet	30 mins–3.5 hours	297–300

Table 2 *(Contd.)*

Technique name	Description	Sample types and processing	Current application maturity and limitations	Time needed[a]	Example references[b]
Optical biosensors	Fluorometric or colourimetric biosensors, can be integrated with smartphone cameras; SPR detects real-time changes in refractive index upon target analyte binding and bacterial responses to antimicrobials.	Clinical isolates, clinical samples (blood, urine), environmental samples (water); filtration, addition of indicator molecule	Commercialized fluorometric and colourimetric products, SPR in advanced research stages; scattering effects, instrument costs, limited miniaturization due to optical components	1–6 hours	301–303
Piezoelectric biosensors	Quartz crystal microbalance (QCM) biosensors report changes in frequency signals when target analyte or probes are bound to the biosensor.	Clinical isolates, bacterial isolates; filtration	Successes in proof-of-concept research; specialized instrumentation, sensitive to matrix effects requiring pre-processing samples	20 mins–4 hours	304 and 305

[a] Time of the detection technique, does not include obtaining bacterial isolates in which 18–48 hours is added to the testing time. [b] Non-exhaustive list.

the high frequency of antimicrobial use, combined with the presence of immunocompromised individuals and complex medical interventions, creates ideal conditions for resistant organisms to thrive.[29] The consequences place a substantial burden on healthcare systems, with increased infection rates, higher morbidity and mortality, extended hospital stays, and rising demand for specialized care.[307] In Canada, resistant bacterial infections currently impose an annual cost of $1.8 billion on the healthcare system, a figure expected to rise to over $8 billion by 2050 without intervention.[9] In a multicentre cohort study of 12 000 patients across 66 countries, 21.6% of gastrointestinal surgical site infections were caused by organisms resistant to the prophylactic antibiotics administered perioperatively. The proportion of resistant infections varied substantially by national development level, ranging from 16.6% in high, 19.8% in middle, to 35.9% in low-income settings, highlighting significant disparities in the effectiveness of standard surgical prophylaxis globally.[236,308] Beyond economic strain, the persistence of resistant pathogens fundamentally threatens the viability of medical procedures that rely on effective prophylaxis, from chemotherapy to organ transplantation.[21] In a 10-year study conducted in France between 2001 and 2010, 4.1% of the 710 liver transplant recipients were found to be colonized with extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* prior to transplantation. Within four months postoperatively, 5.5% of patients developed ESBL-related infections, with intra-abdominal infections being the most common type of infection.[309,310] In 2022, the WHO identified 19 fungal pathogens as priority for research and development to address the rising fungal AMR public health threat.[97] The WHO lists yeasts *Candida auris* and *Candida albicans* as two of four fungi identified as a critical priority, both of which have shown multi-drug resistance.[97] As resistant infections continue to challenge infection control practices and therapeutic protocols within hospitals, understanding their origins, transmission pathways, and persistence in clinical settings remains essential for guiding effective interventions.

Wastewater

Wastewater systems represent a critical link between human activity and the environment, and they play a central role in the mobilization and dissemination of AMR.[90] Antibiotics and ARB, along with ARGs, enter wastewater through multiple pathways. In clinical settings, hospitalized patients often receive doses of broad-spectrum antibiotics that lead to the low excretion of both unmetabolized compounds and resistant bacterial populations *via* urine and feces.[85] These residues and microbial communities enter hospital wastewater streams, contributing significantly to the overall AMR burden in municipal systems.[80,81,311] Surveillance data from France reflect this clinical-environmental interface, where the incidence of ESBL-producing *Enterobacteriaceae* in hospital settings increased fourfold between 2002 and 2013. During this period, *E. coli* became the predominant ESBL-producing species, rising from 19% to 59% of isolates. In parallel, com-

munity-based data indicated that ESBL-producing *E. coli* accounted for an estimated 3–5% of urinary tract infections, with approximately 5% of adults and children identified as asymptomatic carriers by 2011.[84,312–314]

In parallel, communities contribute to the resistome through outpatient antibiotic use. Antibiotics taken at home – often incomplete or prescribed unnecessarily – are similarly excreted, and their residues, along with the resistant bacteria selected in the gut microbiota, enter the domestic sewage system.[315,316] As a result, wastewater systems accumulate inputs from both clinical environments and broader community-level antimicrobial use, making them hotspots for AMR gene exchange and selection under sub-inhibitory antibiotic concentrations.[42,94,317]

WWTPs are not currently designed to target or remove antimicrobial compounds or resistance genes.[93] Conventional treatment processes, including primary sedimentation, activated sludge, and secondary clarification, are effective at reducing organic load and pathogens to meet general effluent quality standards and can remove ARGs and ARBs[318–320] but, they fall short in mitigating the dissemination of ARGs and ARB.[51,91,95,96,235,321–325] These conventional wastewater treatment processes do not specifically target genetic material or residual antimicrobials, allowing ARGs to persist throughout the treatment line.[262] Moreover, the conditions within WWTPs – such as dense microbial communities, nutrient-rich environments, and selective pressures from residual antibiotics and disinfectants – can facilitate the horizontal transfer of resistance genes.[41,42] As a result, effluent discharged following conventional treatment, despite meeting established quality standards in high-income settings, may still contain quantifiable levels of antibiotic residues, AMR bacteria, and ARGs.[176,326,327] These are discharged into receiving water bodies, where they contribute to the environmental resistome and may interact with human, animal, and ecological systems.[119] Additionally, sewage sludge, which is often applied as agricultural fertilizer, represents another route through which resistance elements re-enter the terrestrial environment.[328] In a comparative metagenomic analysis by Bombaywala *et al.* (2021), pharmaceutical sediment exhibited the highest proportion of ARGs (2.52%), followed by effluent treatment plant (ETP) sludge (2.28%) and wetland sludge (1.29%).[222] While pharmaceutical discharge sites contained clinically important ARGs such as *tetA, tetC*, and *qnrS*, the ETP sludge was enriched with multidrug efflux pump genes, highlighting the persistence of resistance elements even in treated waste streams.[222,329]

The growing recognition of wastewater as a major reservoir and conduit for AMR highlights the urgent need to reconsider current treatment paradigms. Addressing AMR in wastewater requires the development of advanced treatment technologies, improved surveillance, and a stronger policy framework that includes resistance as a water quality parameter. Integrating One Health principles into wastewater management is essential to limiting downstream risks and reducing the environmental amplification of resistance.

Septic tanks

Decentralized wastewater systems, such as septic tanks, present distinct challenges in the context of AMR. In rural and suburban areas, an estimated 25% of the population relies on septic systems for domestic wastewater management.[330] These systems function by separating solids and scum from wastewater and allowing the remaining effluent to filter through soil. While they are effective at reducing conventional pollutants, they are not designed to eliminate antibiotics, ARB, or ARGs.[331] The presence of these contaminants in effluent can result in their infiltration into nearby groundwater sources, particularly when systems are poorly maintained or located near wells and boreholes.[332,333] Given that human excreta are estimated to contain 10^{11} bacterial cells per gram of colonic content,[334] it is suggested that the pathogen loads discharged from septic tank effluents may be comparable to, or exceed, infectious dose thresholds capable of initiating disease in exposed populations.[335] Empirical data have shown that faecal coliforms and *E. coli* in septic effluents can reach concentrations ranging from 10^3 to 10^8 CFU per 100 mL, with survival in groundwater extending up to 30 days for coliforms and over 100 days for *E. coli*.[321,331] Since private wells are not subject to regulatory oversight and are often used without prior microbiological treatment, they may serve as an unrecognized route of exposure to resistance-related contaminants, such as ARBs and ARGs.[322] Environment and climate change Canada has identified septic systems as a major contributor to groundwater contamination across the country.[235] Supporting this concern, a 2019 study conducted along a 1.7 km stretch of Lake Huron shoreline in Grand Bend, Ontario, detected artificial sweetener acesulfame in 100% groundwater samples (7–842 ng L^{-1}, n = 78), confirming widespread contamination of the surficial aquifer by septic system effluent.[323] Nitrate levels (3.5 ± 1.4 mg L^{-1}) were positively correlated with acesulfame (r^2 = 0.54), further implicating septic systems as a substantial source of nitrogen loading to groundwater.[323] These findings illustrate how septic effluent can influence drinking water quality even when systems are functioning as intended.

Agricultural and veterinary

Antibiotics are widely (mis)used in all areas of industrial agriculture including intensive crop cultivation, livestock farming and aquaculture to treat infections, prevent disease prophylactically, and promote growth.[2,66,336–338] The rise of AMR in the agricultural sector is detrimental to the health of the crops and animals, causing losses for farmers and consumers. Food animals (terrestrial and aquatic species) farmed as monocrops under crowded and stressful conditions of industrial agricultural practices are prone to infections.[66,203,338,339] These conditions increase the exposure risks and transport of AMR into other areas such as into the food industry and to the consumer, or into the environment through runoff or leachates.[70,175,338,340–343] Leachates can also enter environmental waters, into the water table, and further to groundwater.[70,235,237,338,344] Antibiotics are commonly distribu-

ted to livestock in their feed resulting in potential release of antibiotics directly into the local environment risking AMR evolution. The microbiomes within these animals experience constant exposure to antibiotics risking the selective pressure that favours the evolution of AMR. Animals infected with resistant microbes risk spreading AMR between animals and humans through bacteria such as *E. coli* which are shared pathogens.[66,338,343]

Animal livestock

Antibiotic use has been prolific in livestock farming since industrialization. To capitalize on production, factory farming practices, such as maximizing the number of animals in one space, result in sanitary issues and higher rates of disease transmission between animals and ultimately to humans.[339] The World Organization for Animal Health (WOAH) estimated that up to 88 927 tonnes of antimicrobials were consumed for veterinary purposes of food-producing animals (includes aquatic animals) globally in 2021.[345] An analysis from Ardakani *et al.* (2024) determined an estimate of 76 060 tonnes for global annual use of AMAs over 2019–2021 for cattle, chicken, and pigs.[346] Analyses show that most antimicrobial agent usage by biomass is in bovine farming. While there are data availability limitations that would affect the calculations methodology, of which were reported by authors, the significant tonnes of antimicrobials (mis)used in livestock agriculture is a high-risk driver of AMR.[346]

Additionally, the faeces and urine wastes of these farm animals will also contain antibiotics residues, ARGs, and AMR microbes.[66,71,73,343,347–350] Wastes from livestock can be purposefully used in other agricultural applications such as manure application for fertilization in plant agriculture resulting in another environmental contamination pathway.[66,70,71,73,95,95,338,343,344,347,348,350] These wastes result in another exposure mechanism pathway into the environment through soil contamination which could percolate to the water table and contaminate agricultural runoff from weather.[66,70,328,338,344,350] The accumulation overtime of AMR in these soils, particularly in fields where manure is applied annually, results in a persistent AMR contamination and HGT mechanisms allow for the proliferation of AMR among the indigenous environmental bacterial communities.[70,71,73,95,328,333,347,348,351,352]

Plant agriculture

Plant agriculture is less commonly associated with antibiotics and AMR in agriculture but contribute significantly to environmental AMR through multiple pathways. Plant pathogens including fungi, bacteria and viruses cause infectious disease, such as blight, that can destroy crop yields. Antibiotics like streptomycin and tetracyclines are commonly used in plant agriculture for disease control for their broad-spectrum activity against both gram-negative and gram-positive bacteria. This drives AMR in relevant plant and crop pathogens, such as documented streptomycin resistance in *Erwinia amylovora* that causes fire blight in apples and pears and *Pseudomona syringae* that causes cankers, but also in non-pathogenic microbes in the soil and foliage.[184,204,261,353–355] This inadvertently creates ARG reservoirs in these soil bacteria which can transfer to animal and human pathogens *via* HGT. Soil contaminations such as metals, pesticides, and other organic pollutants would also exert selective pressures on these microbial communities as discussed previously. All the extracellular AMR DNA persist in the soil environment due to the ability of the DNA to adsorb onto the surfaces of particles, such as clay and sand, that make up soil. DNA/RNA extraction is required for soil samples but the adsorption of DNA to the particulate matter decreases the efficiency of DNA extraction.[356]

Plant agriculture AMR can be exacerbated by any AMR contamination from manure and/or sludge applications as fertilizers that may contain many AMR microbes and ARGs.[66,71,338,348,351] Manure and sludge provide a nutrient-rich environment for diverse microbes that can facilitate HGT of ARGs. Unmetabolized antibiotics excreted in livestock waste create sustained selective pressure on soil and plant-associated microbes to develop AMR.[66,71,73,338,343,347,348,350,351] It has been observed that manure application influences the indigenous soil microbiome more than chemical fertilizer, including increasing the diversity and abundance of ARGs and MGEs. Repeat yearly manure applications provide more nutrients and potentially ARGs to the soil microbiome that would stimulate growth of the soil microbiome including the ARB.[66,71,328,333,338,344,347,348,350,351] A unique factor of sludge being a product of human sewage waste results in introducing clinically relevant microbes or ARGs to the soil microbial community.[95,158,328,352] A study on postdigestion sewage sludge in Ontario found that the AMA triclosan was the predominant contaminant applying selective pressure to microbes among 100+ other compounds.[158] Although further treatment of these waste products may reduce AMR microbe or ARG presence, providers often fail to adequately treat them prior to transport and field application, risking worker and environmental exposure.[73,166,328,329,357] Farmed crops ultimately serve as pathways for AMR and resistant microbes into the food chain.

Aquaculture

Aquaculture is a growing agricultural practice as global fishery yields decrease with increasing demand for aquatic proteins and delicacies.[337] According to the Food and Agriculture Organization (FAO) of the United Nations, aquaculture produced 51% of global aquatic animal production in 2022, representing 94.4 million tonnes, surpassing fisheries production for the first time.[337] This presents a significant source and transport mechanism for AMR because of the nature of aquaculture and the ability to enter the food chain. Aquaculture systems are unique in that the production infrastructure is directly within and surrounded by wild aquatic ecosystems. Being inside of the larger aquatic or marine environment, there is an additional consideration for the ubiquitous presence of microplastics and metals in the water. As previously described, microplastics offer high surface area substrates for

biofilms to develop on and bioaccumulate in the food chain *via* ingestion by the aquatic animals.[137,140,145–147,337]

Aquaculture uses the same principles of maximizing the number of animals as monocrops within a given space. These crowded and stressful environments give rise to vulnerability to disease.[58,146,337,339,341] Like livestock agriculture, the antibiotics are provided through the animal feed which must be put directly into the aquaculture open nets that are in the lake or ocean ecosystem. This results in a direct addition of antimicrobials and AMR to the environment and the wild aquatic life. Interactions of the AMR microbes in the wild environment, such as HGT or farmed and/or wild animal infection, facilitates the evolution, spread, and exposure of AMR across many environments over large geographical areas.[58,146,337,339,341] There are also climate considerations as warmer water temperatures can facilitate pathogen growth and disease outbreak in the aquatic animal and/or algae monocrops.[223,224,337]

Waste and landfills

Pollutants from landfills can often be released into the local environment, such as *via* leachates, resulting in landfills being a potential long-term source of AMR and particularly ARGs.[72,87,209] Landfills receive hundreds of millions of tonnes of solid waste containing food and other organic pollutants, metals, plastics, personal care products, and pharmaceuticals, including antibiotics.[209,216,358] These wastes and pollutants collectively exert selective pressures on the microbial communities over decades. Depending on the composition of wastes and environmental conditions (water, nutrients, oxygen, pH, *etc*), effectively creating microenvironments, the spatio-temporal distribution and proliferation of AMR and ARGs could vary dramatically.[61,87–89,209,216,225,260,359] This results in very heterogenous samples and microbial communities that are dependent on consumerism and other local anthropogenic activities. For example, Threedeach *et al.* (2012) tested the susceptibility of 80 *E. coli* isolates from leachates of anaerobic and semi-aerobic landfill systems to 31 antibiotics using the Kirby–Bauer disk diffusion test. Their results found that over 80% of *E. coli* from both leachate types were found to be resistant to one or more antibiotics and that generally the isolates from the anaerobic leachates had higher percentage of resistances.[89]

Extensive landfill studies have been completed in China including in the context of AMR. Song *et al.* (2016) found that sulfamethoxazole, tetracycline, and oxytetracycline antibiotics were abundant in refuse samples obtained from different areas and depths in a large landfill in central China. The related ARGs (*sul*1 and *tet*O) were detected, with large variation, in all their 51 refuse samples using qPCR.[360] These genes were positively correlated with sample moisture content of which Song *et al.* suggest that ARGs may be proliferated or transported with water in landfills. Wu *et al. (2017)* conducted a comprehensive study of a landfill in Shanghai, China with various leachate and refuse samples taken at different ages of the landfill.[87] The study quantified four classes of antibiotics (tetracyclines, sulfonamides, macrolides, and β-lactams) with UPLC-MS/MS, metals (Al, As, Fe, Ni, Cr, Cu, Mn, Pb, Zn, Cd, and Co) with ICP-OES, 12 ARGs and 6 mobile genetic elements (MGEs) *via* qPCR, as well as other physicochemical properties such as pH and content of N and P. Wu *et al.* (2017) found that all metals tested, except for Co, were found in all their leachate and refuse samples. Their study revealed trends where antibiotics concentrations decreased, metals concentrations increased, and concentrations of ARGs and MGEs increased with landfill age in leachates.[87] The positive correlation of increasing metals concentrations and increasing ARGs in leachates with landfill age suggests a co-selective pressure on the landfill microbial communities[87,88,225,226,359] of which Wu *et al.* (2017) determined their samples were composed of at least 23 phyla.[87] A study by Wang *et al. (2022)* used qPCR and Illumina sequencing to analyse 56 leachate samples collected from seven representative landfills in China. It was determined that 1210 known ARGs were identified, including several multi-drug ARGs such as *Emr*B-*Qac*A, *mdt*E, *mdt*L that encode multidrug transporters and efflux complexes. Alarmingly, pathogenic species *Salmonella enterica*, *Labilibaculum filiforme*, Bacteroidales *bacterium*, *Anaeromassilibacillus senegalensis*, and *Pseudochrobactrum* sp. were identified as ARG carriers.[88] There may also be missing information in their data given that the leachates that were centrifuged and the DNA extractions were performed on the pellet only with a soil extraction kit while the supernatant was discarded.

Several landfill studies show that concentrations of ARGs increased with landfill age[87,216,260,360,361] while others show a negative correlation.[225] The innate variability in landfill samples due to localized anthropogenic behaviours, waste types, and biogeochemical differences make it difficult to determine trends in the abundance, fate and transport of AMR and ARGs in landfills without large-scale international co-operative spatio-temporal studies. For example, the differences in abundance of ARGs in leachates and refuse with plastics is that there are a high presence and continuous production of microplastics in landfills, and any attached AMR biofilms, that could be in samples.[51,123,135,278] It has also been shown that bacteria may selectively colonize some substrates like microplastics more than wood or glass with factors like surface area and surface charge affecting selectivity.[63,138] The quality of landfill refuse samples could vary given that analytes must be extracted from portions of heterogenous solid wastes at various stages of decomposition which may first need to be reduced in size *via* homogenization. The composition of refuse samples then must be characterized to interpret results appropriately and multiple samples should be taken from various locations and depths in the landfills.

Another transport mechanism that can complicate the issue of AMR in landfills is through bioaerosols released from landfill sites due to various waste disposal activities and local environmental conditions.[205,208,209] For example, a worker could be transporting and unloading a truck full of waste to a landfill site and releasing small particulate matter, dust, and bioaerosols into the air. If there is not appropriate personal

protective equipment for the worker, bioaerosols can enter their respiratory tract. Due to the heterogeneous and complex microbial community in different parts of landfills, the bioaerosols are thus also quite complex and could include microbial toxins, viruses, dust, ARGs, and possibly cells or parts of colonies that are attached to the small particulate matter. The released bioaerosols can result in health impacts for the landfill employees and any nearby communities of microbes, animals, and humans.

While modern landfill sites manage the collection and decontamination of leachates, ARGs and AMR microbes have been shown to persist in treated leachates. Thus, there are still risks of leachates entering the local environment, surface waters, and potentially into the water table *via* infiltration.[86,88,89,148,148,209,225,226,232,260,362] A recent study by Jia *et al.* (2024) used metagenomic sequencing to determine the antibiotic resistome of raw and treated leachates and in the groundwater surrounding three landfills where ARGs were found in all samples. Human pathogens *Acinetobacter pittii*, *Pseudomonas stutzeri*, and *Pseudomonas alcaligenes* were found to be carriers in the leachate and groundwater samples.[232]

Groundwater

Groundwater is a key source of drinking water in many regions, particularly in rural and urban areas. In the US and Canada, groundwater serves approximately 43 million and 3–4 million people, respectively.[322,363] While typically perceived as a protected resource, growing evidence suggests that groundwater can act as a reservoir for antibiotics, ARBs, and ARGs.[19,237] These contaminants may enter aquifers through several pathways, including leaching from manure-amended soils, infiltration from poorly maintained septic systems, and surface runoff from agricultural and wastewater impacted sites.[70,364] A study in the Republic of Ireland looking at private wells found that all *E. coli* isolates displayed resistance to at least one veterinary antibiotic, with 93% resistant to aminoglycosides, while 21.4% were resistant to human-use antibiotics.[365] Similarly, a Canadian cross-sectional study of 878 individuals reported that those consuming groundwater contaminated with AMR *E. coli* were 26% more likely to be colonized by resistant strains, with 41% carrying resistant *E. coli* and 28% carrying multidrug-resistant isolates.[121] Once introduced, antibiotics and resistance elements may persist in subsurface environments due to limited degradation processes and relatively stable physicochemical conditions. Microbial communities in groundwater systems can further facilitate the survival or horizontal transfer of ARGs, contributing to the environmental resistome. Areas near septic tanks or intensive agricultural activity are vulnerable, especially where private wells are used without regulatory oversight or microbiological treatment.[233,237,366,367]

The persistence of AMR-related contaminants in groundwater presents a direct exposure risk to humans and animals.[66] Furthermore, the remediation of contaminated aquifers is technically challenging and often cost-prohibitive, highlighting the importance of upstream controls. Recognizing groundwater not only as a receptor but also as a potential long-term reservoir for AMR highlights the need to further investigate its role within broader environmental transmission pathways.

Across these environments, AMR does not arise from isolated sources but from a web linking clinical practice, community antibiotic use, waste management, agriculture, and environmental reservoirs. A systems-thinking, One Health lens therefore requires analytical designs that treat hospitals, wastewater and septic systems, agricultural and aquaculture operations, landfills, and groundwater not as separate case studies, but as interconnected nodes within a shared AMR network, where signals can be traced along pathways. By aligning sampling strategies, locations, co-contaminant measurements, and data integration across these sectors, AMR surveillance can move beyond compartmentalized monitoring and toward an integrated analytical system capable of capturing emergent resistance dynamics to inform coordinated, cross-sector interventions.

Conclusions, synthesis, and recommendations towards an integrated One Health approach

This review presents the current progress in AMR detection technologies and AMR research that inform response initiatives such as monitoring programs. This review also presents many of the significant knowledge gaps and challenges in the global approach to addressing AMR. The multidisciplinary, multisectoral, complex, interconnected issue of AMR requires a response that is just as multifaceted in its international cooperation. However, the current research, monitoring, and policy remain in separate sectors and samples are analysed without analysing other co-contaminants or consider how co-contaminants effect AMR analysis despite the widespread recognition of the interconnected nature of AMR. This disconnected approach does not reflect real-world samples and the reality of the complex and dynamic AMR problem, reflecting a limitation to effective AMR mitigation.

Main limitations of the existing system

Compartmentalization of AMR issues has resulted in differences in methodology and language which prevents meaningful comparisons across sectors ultimately creating inconsistent messaging to all stakeholders. The consequences extend beyond academic inefficiency. When AMR surveillance systems operate independently across human, animal, and environmental health sectors, we lose important insights about AMR transport and transmission pathways. For instance, the role of environmental reservoirs like landfill leachate, wild animals, and WWTPs in AMR dissemination and transmission is poorly understood and is not factored into broader resistance monitoring frameworks. To address the knowledge gaps in understanding AMR and any methodological inconsistencies com-

prehensively across sectors, stakeholders, countries, *etc*, mass data collection and data sharing must be a priority. Many of the technologies described in Table 2 showed capability to achieve results within hours instead of days and potential for portable systems, but these methods are not yet ready for reliable, rigorous, real-time monitoring. Thus, there is an urgent demand for innovative detection technologies and infrastructure to collect real-time data to monitor and reveal spatiotemporal trends across different environments and sample types.

Specific recommendations for analytical decisions and design

The AMR-interacting co-contaminants and key environmental case studies discussed in this review highlight that AMR emergence and dissemination are shaped by interconnected sources, pathways, and co-contaminants across human, animal, and environmental systems. To translate this systems-understanding into practice, analytical scientists need concrete design principles for sampling, measurement, and data integration. The following points summarize specific analytical considerations that can guide the development of AMR monitoring systems capable of capturing these complex dynamics and support more effective interventions:

- Multi-matrix compatibility. Designing extraction methodologies and/or new sensing technologies to analyse AMR across various sample matrices such as environmental waters, wastewater, soil, mixed-materials refuse, *etc*. This would also address heterogenous sample matrices.
- Multi-analyte considerations to account for co-contaminant and co-selection of AMR in key environments. For example, monitoring methodology in environments such as animal agriculture should consider measurements of veterinary antibiotics, metals, and pesticides in water, soil, and manure samples to interpret AMR data in context.
- Sampling across key interconnected environments. Design AMR monitoring networks to include key locations where different environmental sectors intersect to allow for transport pathway analysis. Including the location, sampling depth, and sampling time metadata could help with pooled spatiotemporal analysis.
- Design for data comparability and integration with standardized cross-sectoral AMR indicator targets and units reporting. Determine routine genotypic and phenotypic data that must be reported across human, animal, and environmental surveillance to allow for quantitative comparisons and integrated datasets.

Suggestions for the future at a global structural context

The threat of AMR and its ease in transport must drive investment in research and development of new detection technologies, but also remove policy, detection, and treatment implementation barriers for all peoples internationally, particularly in low- and middle-income communities. The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) has expanded from 25 participating countries in 2016 to 104 countries in 2023. However, 48% of countries did not report data to GLASS in 2023, and approximately half of reporting countries still lack the system infrastructure to generate reliable data.[368] Despite recognition of AMR as a global health priority, there is a discrepancy between statements of priority and research funding investments. A critical barrier exists between research and policy implementation as a vicious cycle: insufficient research funding leads to incomplete understanding of AMR dynamics, which in turn provides policymakers with justification for inaction. AMR research requires long-term investments to generate meaningful results, and the complex global nature of AMR requires coordinated international research and responses.[16,103,368,369]

This level of cross-sectoral, international cooperation in research, knowledge sharing, and implementing responses would require a restructuring of politics and our current segregated competitive systems and funding agencies that results in transnational collaboration, global solidarity, and collective care. This can look like building the research and operations infrastructure and developing a standardized international AMR stewardship program that educates operators and the public for successful autonomous operations, particularly in low-resourced areas to set up everyone for successful AMR responses. It could look like ensuring treatment for infections are accessible as well as developing and educating about new vaccines to prevent infection.

The high costs of AMR in healthcare, manufacturing antibiotics, agricultural and economic productivity, and human and animal lives are known.[15,16,97,236,368,369] Decreasing the prevalence and impact of AMR would reduce those costs, where monetary savings could be redistributed to other government departments that directly benefit the people. However, the response to AMR must not be driven by money or profit where new technologies are inaccessible or render AMR monitoring and stewardship programs inaccessible. Rather, it must be driven by our social responsibility to uphold fundamental human rights, and in particular, human rights to clean water, well-being, medical care, education, and the right to share in scientific advancement and its benefits, as outlined in articles 26–29 in the Universal Declaration of Human Rights by the United Nations.[370] AMR must also be considered when working to address the United Nation's SDGs. While AMR is relevant to all SDGs, the importance of water to human and environmental life and health and consequently has implications on global economic productivity and social inequality, AMR is directly linked to and can be addressed specifically in the following SDGs:[15]

(1) No poverty – an additional 28.3 million people could be pushed into extreme poverty by 2050 due to chronic AMR infections and increasing treatment costs making treatment for the poor inaccessible.

(2) Good health and wellbeing – chronic infections and deaths by AMR will increase.

(3) Clean water and sanitation – lack of access facilitates spread of disease and increases morbidity and mortality while the environment has also become a reservoir for AMR.

(4) Reduced inequalities – chronic infections and increasing treatment costs will increase inequalities in already vulnerable groups such as racial and gender minorities, children, people employed in sectors like healthcare or agriculture, and people living in poverty

(5) Responsible consumption and production – misuse of antibiotics in agricultural and clinical contexts and antimicrobial compounds released from wastewaters of pharmaceutical companies and healthcare sites pollute the environment and promote AMR, food safety and security is increasingly threatened by AMR.

(6) Climate Action – global warming has resulted in increased frequency and severity of extreme weather events which along with forced migration spread AMR.

(7) Life Below Water – aquatic environments have become reservoirs for AMR and misuse of antimicrobials in aquaculture promote AMR.

(8) Life on Land – antimicrobial pollution and AMR spread, and reservoirs such as soils and animals change ecosystems and decrease biodiversity.

(9) Partnerships for the goals – transnational, interdisciplinary, cross-sectoral, collaborative partnerships and research and development is required to effectively address AMR.

AMR within this SDG and human-rights context reinforces the systems thinking and One Health lenses in that analytical systems, surveillance strategies, and technological investments must be designed not only for analytical performance, but also for accessibility and equity to ensure that the science is for the people. AMR research has previously conducted within sectoral silos, and it is important that this research continues to obtain more data and deeper understandings of AMR in different contexts. However, the underestimated complex role of the environment and vast number of interconnected transport pathways between and within sectors call for urgent expansion into interdisciplinary transnational collaborative research and systems thinking guided by the One Health Framework. Only a coordinated global response, informed by the inclusion of Indigenous knowledge keepers and scientists, will result in equitable comprehensive meaningful change in addressing the worsening AMR crisis and protect present and future generations for all.

Author contributions

Rebecca X. Y. Chen: writing – review & editing, writing – original draft, visualization, conceptualization. Rayane Azani: writing – review & editing, writing – original draft, visualization. Kayla Elliot: writing – review & editing, writing – original draft. Sarah Jane Payne: writing – review & editing, writing – original draft, conceptualization. R. Stephen Brown: writing – review & editing, writing – original draft, supervision, conceptualization. Zhe She: writing – review & editing, writing – original draft, supervision, conceptualization.

Conflicts of interest

All authors confirm there are no conflicts to declare.

Data availability

No primary research results and original or new experimental data was generated or analysed as part of this review article.

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