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Critical Review

The Complex Interplay Between Antibiotic Resistance and Pharmaceutical and Personal Care Products in the Environment

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Abstract: Antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are important environmental contaminants. Nonetheless, what drives the evolution, spread, and transmission of antibiotic resistance dissemination is still poorly understood. The abundance of ARB and ARGs is often elevated in human-impacted areas, especially in environments receiving fecal wastes, or in the presence of complex mixtures of chemical contaminants, such as pharmaceuticals and personal care products. Self-replication, mutation, horizontal gene transfer, and adaptation to different environmental conditions contribute to the persistence and proliferation of ARB in habitats under strong anthropogenic influence. Our review discusses the interplay between chemical contaminants and ARB and their respective genes, specifically in reference to co-occurrence, potential biostimulation, and selective pressure effects, and gives an overview of mitigation by existing man-made and natural barriers. Evidence and strategies to improve the assessment of human health risks due to environmental antibiotic resistance are also discussed. *Environ Toxicol Chem* 2023;00:1–16. © 2022 SETAC

Keywords: Wastewater; horizontal gene transfer; environmental contaminants; risk assessment

INTRODUCTION

Antibiotics, antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and coselecting agents are globally released from animal wastes and wastewater to the environment. However, the magnitude of such releases, and their impact on the spread of antibiotic resistance varies widely from place to place, depending on the existing infrastructure and the governance of wastes at local scales (Collignon et al., 2018; Medlicott et al., 2020; World Health Organization [WHO], Food and Agriculture Organization of the United Nations [FAO], World Organization for Animal Health [OIE], & United Nations Environment Programme [UNEP], 2022). In general, healthcare systems in low-to-medium income countries are much more affected by such releases than are those in high-income countries (Malchione et al., 2019), although factors that promote the development, transmission, and spread of antibiotic resistance exist in all systems.

The influence of pharmaceuticals (including antibiotics) and personal care products (PPCPs) on the development and spread of antibiotic resistance, within both waste streams and receiving environments, is still poorly understood. Evidence indicates that

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1) in some cases ARGs may confer a fitness advantage to their bacterial hosts (i.e., the capacity of host bacteria to grow and outcompete others) at environmental antibiotic concentrations that are below the minimum inhibitory concentration (the concentration above which bacteria cannot grow), or even in the absence of known selective pressure (Murray et al., 2021); 2) individual nonantibiotic chemical contaminants, or complex mixtures of PPCPs, may influence the dissemination of ARB or ARGs by triggering horizontal gene transfer or cell-defence mechanisms (Maier et al., 2018; Wang et al., 2019, 2020); and 3) humans, animals, and the environment are part of a single antibiotic resistance dissemination path—the One-Health spectrum (Van Bruggen et al., 2019; WHO et al., 2022). However, it is unclear to what extent and under what conditions antibiotics and other potential coselecting chemicals and/or agents can impact resistance development, transmission, and/or spread. Filling this knowledge gap is critical for developing holistic mitigation strategies within a One-Health agenda.

Antibiotics have been used since the 1940s in human and animal medicine to treat bacterial infections (Davies & qDavies, 2010). However, they are also extensively used worldwide as animal growth promotors and prophylactic agents (McEwen, 2006). Multiple studies published in the past 20 years have provided evolutionary insights into the environmental origins of ARGs currently causing problems in healthcare settings (Larsen et al., 2022). The environmental resistome (the collective of genes conferring resistance) mixes with human- and animal-associated bacteria, along with chemical contaminants, which are continuously introduced to natural environments through fecal waste streams (Figure 1). Wastewater treatment plants (WWTPs) can remove one to six log-units of ARGs (per unit volume), but removal values heavily depend on the specific treatment technology used and how well the WWTP is operated (Graham et al., 2018; Manaia, 2022; Marano et al., 2021). In general, large quantities of ARGs, including those associated with pathogens, are still emitted in treated effluents (Manaia, 2022).

On a global scale, this situation is aggravated by the fact that approximately half of the world's population do not have adequate wastewater treatment (Medlicott et al., 2020). Multiple reviews have emphasized the role of antibiotics as major drivers for antibiotic dissemination (Larsson & Flach, 2022; Manaia et al., 2020). In the present review we aim to highlight that antibiotic residues are one of the numerous environmental contaminants that are capable of interfering with microbial communities. We argue that the combined effect of different contaminants, and not only antibiotics, has the potential to favor some lineages to the detriment of others, therefore leveraging the proliferation of some ARB and ARGs. Specifically, our review aims to: 1) present evidence that antibiotic residues co-occur in the environment in combination with a myriad of other contaminants and that it is the whole range of contaminants, rather than antibiotics alone, that are expected to influence the fate of ARB and ARGs; 2) discuss the role of important sources, recipients, and barriers (natural or man-made) where ARB, ARGs, and

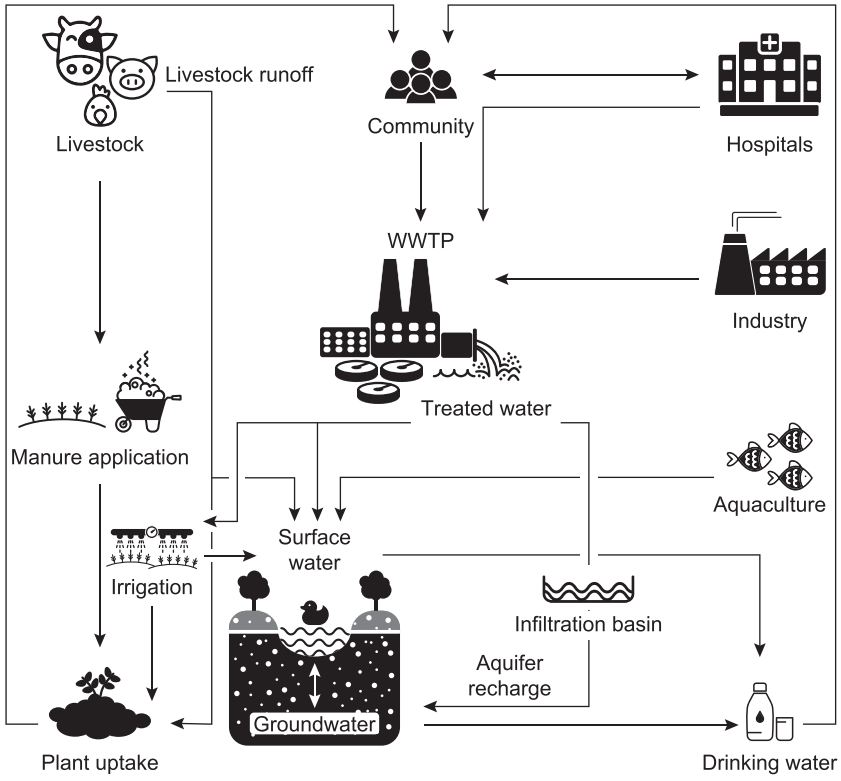

FIGURE 1: Schematic diagram of how antibiotics and other chemical contaminants, antibiotic resistant bacteria (ARB), and their genetic determinants (anbiotic resistance genes [ARGs]) can end up in the human food systems. Water reuse, which is a common practice in arid and semi-arid regions, uses treated wastewater to irrigate food crops; biosolids are typically land-applied as fertilizer. Both practices can introduce chemical residues, ARB, and ARG into food for human consumption. WWTP = wastewater treatment plant.

other contaminants frequently co-occur and how these can be managed to minimize the potential exposure to humans; 3) examine the risks to human health arising from enhanced environmental selection for ARB; and 4) consider mitigation strategies that are relevant to both low-to-medium income and high-income country environments.

CHEMICAL CONTAMINANTS

Chemical contaminants occur as complex mixtures

Antibiotics have been considered the primary driver of antibiotic resistance development and selection in medicine and in agriculture (Davies & Davies, 2010; Larssson & Flach, 2022; Manaia et al., 2020). In many parts of the world, antibiotics are introduced directly into the environment through aquaculture, animal husbandry, and crop production (Figure 1). For instance, the implications of the use of colistin in husbandry and aquaculture are just one example of how this drug contributed to the development of colistin-resistant ARB and the dissemination of ARGs (e.g., *mobilized colistin resistance* [*mcr*] genes) over distinct One-Health compartments (wild animals, food products, humans, and pets; Shen et al., 2020). Many antibiotics do not readily degrade, and some of their metabolites maintain bioactivity or may transform back to the original form (Kümmerer et al., 2019). These drugs are often highly polar, and thus water soluble and amenable to environmental transport. Less polar chemicals will sorb to and be retained by sediments and soil, reducing their potential for environmental mobility. Irrigation with reused wastewater and land application of biosolids and manure will carry chemicals into soils, and mobilization to surface and ground water may also occur (Figure 1). Whereas these open systems can potentially release chemicals without dedicated treatment, WWTPs are essential barriers to attenuate the emissions of many contaminants present in raw wastewater. However, the efficiency of WWTPs to remove a given antibiotic depends on the compound's physicochemical properties, and the design and operation of the treatment system, especially ambient oxidation-reduction conditions (Angeles et al., 2020). The frequent detection of antibiotic residues in aquatic environments is evidence that these contaminants can be released from multiple sources, leading to pseudo-persistence (Fang et al., 2019; Wilkinson et al., 2022).

Booth and coworkers (Booth et al., 2020) have created a systematic database based on the data aggregated by the German Environment Agency (2016) to determine antibiotic residue concentrations that exceed the predicted no-effect concentrations (PNECs) for possible antibiotic resistance selection in various environmental matrices. These include municipal WWTP effluents, industrial wastewater effluents, hospital wastewater effluents, surface water, and drinking water across 47 countries (Table 1). Almost 6% of the samples analyzed contained antibiotic concentrations exceeding their PNEC values. Hospital wastewater (42.8%) and industrial wastewater had the highest concentrations of antibiotic residues, with 47% of the samples exceeding the PNEC values. Drinking water had no antibiotic concentrations that exceeded any PNEC. For the other matrices, ciprofloxacin (34.9%) and enrofloxacin (10.7%) had the highest proportion of analyses that exceeded the respective PNEC values, whereas amoxicillin (0.6%), clindamycin (0.2%), doxycycline (0.6%), and sulfamethoxazole (0.2%) had the lowest proportion of concentrations that exceeded any PNEC. However, PNEC values for antibiotic resistance endpoints must be used with caution because current values are highly contextual. Matrix and ecological factors, rather than only chemical concentrations, may influence resistance selection; this point is discussed further in the following section, *Critical environmentally relevant concentrations of chemical contaminants*.

Although considered potentially as the primary selectors of ARB and ARGs in the environment, antibiotics often occur mixed with many other chemical contaminants, including pesticides, PPCPs, industrial chemicals, metals, and other wastewater- and manure-derived nutrients (N, P, dissolved organic matter; Manaia, 2022). The complexity of the mixtures of chemical contaminants in aquatic systems is illustrated by the recent study of Wilkinson et al. (2022), who measured the levels of 61 active pharmaceutical ingredients in rivers from 104 countries by targeted analysis. The most frequently detected compounds were carbamazepine, metformin, and caffeine. Four compounds (caffeine, nicotine, acetaminophen, and cotinine), were detected across all continents sampled, and several antidepressants were detected in all continents except Antarctica. Remarkably, antibiotics were detected in rivers worldwide, with some compounds (sulfamethoxazole, metronidazole) being among the substances with the highest concentrations, and many above resistance PNECs. Interestingly, several antimicrobials (antifungal and antibiotic) that were targeted in the analysis were not detected in any water samples, such as cloxacillin (a β-lactam), miconazole, and oxytetracycline, despite their high usage. The lack of detection of β-lactams can be attributed to their instability in the natural environment. Likewise, the lack of detection of other antimicrobials may be due to their partitioning in sediments, low extraction recoveries, or poor detection limits in the analytical method used.

The WWTPs are important sources of chemical contaminants discharged into rivers. Table 2 shows the concentration ranges and frequency of detection of PPCPs in the effluents of 10 WWTPs discharged into the Hudson River (Table 3), a major source of drinking water in the State of New York (USA). Analysis of these samples was performed by targeting 41 PPCPs, out of which 26 were detected during the three sampling events (summer, fall, and winter; Brunelle et al., 2022). Psychoactive pharmaceuticals and antibiotics were detected in all WWTP effluent samples, with total concentrations ranging from 646 to 3250 ng/L, and 307 to 1810 ng/L, respectively. The antibiotics azithromycin, ciprofloxacin, and trimethoprim were detected in the WWTP effluents with 90% or more frequency. Psychoactive pharmaceuticals such as bupropion, carbamazepine, citalopram, desvenlafaxine, lamotrigine, primidone, sertraline, and venlafaxine were also detected in 90% or more of the samples. In addition, when the same samples were analyzed based on suspect screening using nontarget analysis (NTA), 50 pharmaceuticals, nine industrial chemicals, and eight pesticides were

TABLE 1: Mean concentration and number of samples analyzed for each antibiotic and environmental matrix across 47 countries (based on Booth et al., 2020)

		Mean concentration (in µg/L)[a]					No. of analyses exceeding PNEC (no. of analyses performed[b])					Proportion of Analyses Exceeding PNEC Globally (%)
Antibiotic	PNEC value	Municipal wastewater	Hospital wastewater	Industrial wastewater	Surface water (river/stream)	Drinking water	Municipal wastewater	Hospital wastewater	Industrial wastewater	Surface water (river/stream)	Drinking water	
Amoxicillin	0.250	0.1	0.1	—	0.0	0.0	3 (243)	0 (3)	—	0 (230)	0 (20)	0.6
Azithromycin	0.250	0.2	0.9[c]	0.0	0.0	0.0	68 (322)	31 (69)	0 (3)	46 (964)	0 (120)	9.8
Ciprofloxacin	0.064	577.6[c]	6.5[c]	3548.6[c]	383.4[c]	0.0	460 (1485)	212 (212)	11 (13)	86 (464)	0 (28)	34.9
Clarithromycin	0.25	0.2	2.8[c]	0.1	0.0	0.0	18 (798)	59 (103)	0 (3)	21 (860)	0 (152)	5.2
Clindamycin	1.000	0.1	0.4	0.0	0.1	0.0	0 (248)	0 (24)	0 (3)	1 (355)	0 (20)	0.2
Doxycycline	2.000	0.2	0.1	0.3	0.0	0.0	3 (194)	0 (15)	0 (2)	0 (266)	0 (44)	0.6
Enrofloxacin	0.064	53.3[c]	1.5[c]	23.0[c]	87.5[c]	0.0	64 (253)	3 (29)	6 (11)	11 (344)	0 (144)	10.7
Ofloxacin	0.500	3.7[c]	4.2[c]	0.5	2.0[c]	0.0	104 (3594)	45 (168)	3 (5)	32 (488)	0 (2)	4.3
Oxytetracycline	0.500	0.1	0.1	23,119.0[c]	0.0	0.0	17 (2625)	4 (21)	17 (28)	0 (473)	0 (40)	1.2
Sulfamethoxazole	16.000	0.3	2.8	18,416,8[c]	0.1	0.0	0 (2833)	1 (170)	13 (32)	1 (2558)	0 (471)	0.2
Tetracycline	1.000	0.2	0.0	453.5[c]	0.0	0.0	8 (294)	0 (57)	13 (26)	0 (491)	0 (165)	2.0
Trimethoprim	0.500	0.6[c]	1.4[c]	3078.7[c]	0.0	0.0	81 (1538)	50 (76)	2 (11)	32 (1437)	0 (329)	4.9

[a]Mean concentration calculated from all database entries that reported the measured environmental concentration as either a mean, median, or single value.
[b]One compound analyzed in one sample equals one "analysis."
[c]Values that exceed the PNEC level for each antibiotic.
Data were extracted from 1016 original peer-reviewed publications and 150 review articles, published up until 2016, that reported environmental concentrations of pharmaceutical substances worldwide in surface water, groundwater, tap/drinking water, manure, soil, sewage, and other environmental matrices (German Environmental Agency, 2016). The table is based on Booth et al. (2020).
PNEC = predicted no-effect concentration.

detected, suggesting that many more contaminants co-occur with PPCPs, including antibiotics, in the environment than are currently monitored in targeted analysis.

The load of PPCPs emitted by WWTPs and discharged by other means into the aquatic systems depends on the socio-economic and consumption patterns in different world regions. This was evidenced in a recent study (Fang et al., 2019) showing that the concentrations of PPCPs were, in general, lower in Europe than in the United States or China. Also, the same study reported that the occurrence of emerging organic pollutants was mainly associated with land use (Fang et al., 2019). These contrasts may provide insightful data about the potential influence of PPCPs as triggers for the spread of ARB and ARGs. To this end, systematic and broader data sets as well as interventions would be needed, moving from research to action. For instance, new applications of NTAs based on high-resolution mass spectrometry and integration of high-performance computing (Singer et al., 2016) demonstrated high potential for real-time monitoring of micropollutants to evaluate treatment efficiency and pollutant prioritization efforts. The use of better management practices that reduce nutrient inputs into watersheds has the cobenefit of reducing micropollutant inputs (Duan et al., 2021; Guardian et al., 2021). Improved monitoring of aquatic systems might require NTA for increasing the chemical coverage of analytical methods, and the use of passive samplers that can extract chemical contaminants with a wide range of polarities. (Textbox 1).

Critical and environmentally relevant concentrations of chemical contaminants

One critical issue related to the evolution of antibiotic resistance is the establishment of the levels of antibiotics or other pollutants that may facilitate the development and selection of antibiotic resistance. Determining relevant concentrations is important if standards are to be implemented by authorities through dedicated policies or at the time of evaluating risks that pollutants pose to the environment. The protection of natural microbial communities in the environment is not currently included in environmental risk assessments (Brandt et al., 2015), even though ecotoxicological effects may not be sufficiently informative to underline the risk of antibiotic resistance development. An updated approach that also incorporates the risk of antibiotic resistance development is necessary (i.e., PNECs specific for antibiotic resistance; Bengtsson-Palme & Larsson, 2016). In the case of terrestrial impact, those PNEC values are less clear than for the aquatic environment, and thus further adaptations of the terrestrial assessment strategy appear to be necessary. Finally, investigating the effect of mixtures of antibiotics and other chemical pollutants, including transformation products, is necessary to predict and assess the impact of chemical contamination in realistic scenarios.

From the antibiotic resistance perspective, besides possible selection, it is important to also understand the mechanisms that underlie the maintenance and persistence of bacteria and their genes Whether the prevalence or abundance of a resistance

TABLE 2: Comparison of selected targeted analytes determined by targeted SRM method, quantified by isotope dilution (A), and using nontarget analysis, quantified with a 1-point external calibration (B) for three effluent samples (Fall, 2019 HR2, HR7, and HR8)

	Fall HR2			Fall HR7			Fall HR8		
	Targeted	NTA		Targeted	NTA		Targeted	NTA	
Compound	Isotope dilution (ng/L)	1-pt Calibration standard (ng/L)	% Difference	Isotope dilution (ng/L)	1-pt Calibration standard (ng/L)	% Difference	Isotope dilution (ng/L)	1-pt Calibration standard (ng/L)	% difference
Acetaminophen	N.D	12.1	—	N.D	6.9	—	N.D	11.1	—
Acetyl-sulfamethoxazole	462	50.3	161	235	45.3	135	N.D	<1	—
Azithromycin	185	25.9	151	188	15.1	170	351	37.6	161%
Bupropion	239	95.5	86	232	72.7	105	295	123	82
Caffeine	324	141	79	47.3	17.1	93	46.4	18.8	85
Carbamazepine	133	28.7	129	127	18.6	149	80.4	20.8	118
Ciprofloxacin	306	117	89	15.8	9.7	48	116	73.1	46
Citalopram	270	55	132	137	26	136	247	75.3	107
Desvenlafaxine	880	194	128	660	109	143	1060	221	131
Lamotrigine	N.D	12.7	—	117	41	96	266	136	64
Paroxetine	N.D	<1	—	N.D	<1	—	N.D	1.5	—
Primidone	222	50.4	126	81.9	31.9	88	96.3	69.7	32
Sertraline	22.4	4.7	130	55	6.5	158	79.1	21.8	114
Trimethoprim	235	43.4	138	206	52.8	118	188	63.3	99
Venlafaxine	332	128	88	216	64.2	108	282	115	84

Concentrations are shown in ng/L, and percentage differences between the two methods are shown where applicable.
HR = wastewater treatment plant effluent samples discharged to the Hudson River; N.D. = nondetect; NTA = nontarget analysis; SRM = selective reaction monitoring.

mechanism (or its host) will increase, decrease, or remain constant within a population will depend on its relative fitness (Andersson & Hughes, 2011) and other habitat factors such as dissolved oxygen concentrations (Jong et al., 2020). The fitness cost of a resistance mechanism, meaning the additional effort of the cell (e.g., energy costs, increase of replication time) to harbor that resistance mechanism, can vary significantly, depending on the type of mechanism, genetic context, or host background (Kraupner et al., 2020). Fitness can be further affected by ecology, for example, the community context (Klümper et al., 2019) and predation (Cairns et al., 2018). Bacterial predators are almost universally aerobic, which means microbial densities of both donors and recipients of HGT will be most impacted by predation when oxygen is present. Indeed, in wastewater ecosystems gene transfer frequencies are observed to be lower in the presence of oxygen (Jong et al., 2020).

The interplay between abiotic factors and ecology-based mechanisms complicates reliable assessment of the dose-effect of chemical contaminants in the environment on the selection of antibiotic resistance. In general, it can be assumed that high-fitness-cost resistance will be lost within a bacterial population over time, whereas low-fitness-cost resistance can be positively selected in the presence of environmentally relevant selective pressure (Andersson & Hughes, 2011). However, high fitness cost can be reduced through mutations (Andersson & Hughes, 2011), or gene duplication or amplification (Sandegren & Andersson, 2009) that could arise stochastically or be induced through stress-mediated regulatory and transcriptional changes (such as activation of the SOS response; Torres-Barceló et al., 2015). Therefore, the relatively low concentrations of selective compounds that can be found in the environment (Wilkinson et al., 2022) may be sufficient for selecting or maintaining resistance. In this way, chemical contamination may increase human health risk due to greater numbers of ARB even when environmental concentrations are below the selective threshold (Murray et al., 2021; Stanton et al., 2022). Conversely, the effects of the chemical contamination will also differ based on factors like oxygen concentration, which may enrich or suppress ARB numbers, depending on other habitat factors. In this context, an improved understanding of the effects of mixtures of antibiotics and PPCPs in the environment and in situ microbial ecology are essential to develop a valid integrated risk assessment. In this regard, efforts are still needed to improve the methods currently described in the literature (Murray et al., 2021; Textbox 2).

TEXTBOX 1: Mechanisms of antibiotic resistance acquisition

Acquired antibiotic resistance involves two major mechanisms: 1) mutating existing genes, and 2) acquiring new genes from other strains (of the same or different species) through horizontal gene transfer (HGT). In general, HGT comprises conjugation, transformation, or transduction. Conjugation is the transfer of DNA between a donor and a recipient, usually mediated by mobile genetic elements, such as plasmids. Transformation results from direct uptake, incorporation, and functional expression of exogenous DNA from the surroundings. Transduction occurs when DNA is transferred among bacteria through bacteriophages.

TABLE 3: Concentrations (ng/L) of detected targeted analytes for surface water grab samples, as well as average concentrations, maximum concentrations, and frequency of detection of targeted pharmaceuticals in fish tissues (ng/kg)

				Wild-caught fish (ng/kg)								
	Surface waters (ng/L)			Reference site			Hudson River: open river			Hudson River: marsh		
Compound	Reference site	Hudson River: open river	Hudson River: marsh	Average	Max	Frequency	Average	Max	Frequency	Average	Max	Frequency
Acetaminophen	N.D.	N.D.	N.D.	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	2.7	1 out of 14
Acetylsulfamethoxazole	N.D.	4	3.9	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—
Azithromycin	N.D.	N.D.	N.D.	N.D.	N.D.	—	21.5	36.8	3 out of 15	18.5	32.6	2 out of 14
Bupropion	N.D.	2.2	0.6	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—
Caffeine	56.8	156	50.8	18.5	41.2	5 out of 34	12.9	29.1	13 out of 15	13.9	36.8	11 out of 14
Carbamazepine	1	4.8	3.5	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—
Citalopram	N.D.	3.8	1.5	N.D.	N.D.	—	N.D.	0.8	1 out of 15	N.D.	N.D.	—
Clarithromycin	N.D.	N.D.	N.D.	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	0.6	1 out of 14
Desvenlafaxine	N.D.	11.8	3.5	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	1	1 out of 14
Haloperidol	N.D.	N.D.	N.D.	N.D.	N.D.	—	1.1	2.4	7 out of 15	N.D.	1.3	1 out of 14
Iopamidol	N.D.	96.1	37.7	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—
Lamotrigine	N.D.	23	38.1	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—
Risperidone	N.D.	N.D.	N.D.	N.D.	N.D.	—	N.D.	1.2	1 out of 15	N.D.	4.95	1 out of 14
Sertraline	N.D.	0.7	0.3	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—
Tilmicosin	N.D.	N.D.	N.D.	N.D.	N.D.	—	N.D.	8.5	1 out of 15	N.D.	9.7	1 out of 14
Trimethoprim	N.D.	3.4	N.D.	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	12.4	1 out of 14
Venlafaxine	N.D.	5.2	1.8	N.D.	N.D.	—	N.D.	N.D.	—	N.D.	N.D.	—

Compounds without detections were not included in the table (anhydro-erythromycin, amitriptyline, ciprofloxacin, diclofenac, enrofloxacin, enrofloxacin, erythromycin, norfluoxetine, norfloxacin, oxolinic acid, primidone, paroxetine, roxithromycin, sarafloxacin, sulfachloropyrazidine, sulfadimethoxine, sulfamethoxydiazine, sulfamethizole, sulfamerazine, sulfamethoxazole, sulfamethazine, sulfadiazine, spiramycin, sulfathiazole, tylosin). Wild-caught fish and surface waters were collected from the Hudson River at Piermont Pier, as well as a reference site (Flax Pond).
N.D. = nondetect.

TEXTBOX 2: Antibiotic resistance as an environmental contaminant

Contaminant ARB are examples of successful biological evolution, whereby the complex interplay among ARG acquisition, stabilization, and expression in the bacterial genome determines the capability of the respective host to survive and proliferate in a microbial community. These contaminants are mostly emitted, directly or indirectly, by sources related to human and animal excreta, such as sewage, manure, wastewater treatment sludges, and final effluents, among others. The most abundant and widespread contaminant ARB are ubiquitous taxa (e.g., *Enterobacteriaceae*, *Acinetobacter*, *Aeromonas*) that thrive in both the human and animal microbiome and in the environment, with the potential to behave as opportunistic pathogens. The fate of contaminant ARB and ARGs in the environment may be influenced by local selective pressures, ecological drivers of the native microbial community, and permissiveness of the receiving microbiome, among other factors.

Selection and horizontal gene transfer triggered by nonantibiotic pharmaceuticals

The contribution of nonantibiotic pharmaceuticals to the emergence and spread of antibiotic resistance has only recently been considered. Hygiene and healthcare products, such as triclosan or chlorohexidine, have been suggested to influence the development of antibiotic resistance, either through selection or by promoting HGT (Jutkina et al., 2018; Lu et al., 2018). Also, numerous studies have reported that nonantibiotic pharmaceuticals (including but not limited to nonsteroidal anti-inflammatories, antidepressants, and lipid-lowering drugs) not only induce multidrug resistance via genetic mutation, but also promote HGT (Jin et al., 2018; Wang et al., 2019). For example, after 30 days of treatment with a typical antidepressant, fluoxetine, mutation frequency in *Escherichia coli* significantly increased, resulting in enhanced resistance against the antibiotics chloramphenicol, amoxicillin, and tetracycline (Jin et al., 2018). Isolated mutants also exhibited multidrug resistance against β-lactams, fluoroquinolone, aminoglycoside, tetracycline, and chloramphenicol (Jin et al., 2018). Also, nonantibiotic drugs could boost antibiotic-like side effects on the gut microbiome and induce antibiotic resistance through activating efflux pumps (Maier et al., 2018). Moreover, nonantibiotic pharmaceuticals could promote the dissemination of ARGs by stimulating conjugation and/or transformation, as was demonstrated in vitro with commercial (culture collection) strains of *E. coli* or *Pseudomonas putida* (Wang et al., 2020, 2021; Table 4).

The suspected reason for nonantibiotic pharmaceuticals inducing the emergence and spread of antibiotic resistance is that such drugs can exert oxidative and other stress in bacteria. After exposure to nonantibiotic pharmaceuticals, bacteria can produce more reactive oxygen species, which can trigger stress responses and damage cell membranes. The empirical evidence related to oxidative stress posed by nonantibiotic pharmaceuticals has been obtained through direct radical measurements, in conjunction with RNA and protein sequencing (Jin et al., 2018; Wang et al., 2019, 2020, 2021). When researchers compared chemical structures and properties between these nonantibiotic pharmaceuticals and various antibiotics, it was found that several common functional groups were shared. For example, ibuprofen, naproxen, gemfibrozil, and diclofenac, harbor benzene rings and carboxyl functional groups, were also present in some antibiotics (Wang et al., 2021). Further studies are required to verify whether and which functional groups facilitate antibiotic-like effects.

LESSENING THE IMPACTS OF CHEMICAL AND BIOLOGICAL CONTAMINANTS: COMBINING ENGINEERING WITH NATURE

Although chemical contaminants, ARB, and ARGs are frequently found together (Figure 1), in most scenarios it is very difficult to determine whether the chemicals themselves promote or select for ARB and ARGs in the environment or are coincidental. Besides domestic effluents, other important sources of antibiotics and other PPCPs as well as antibiotic resistance come from animal production, both husbandry and aquaculture (Topp et al., 2018). The impacts are mainly caused by runoff and/or discharge or use of solid wastes (manure as well as sludge from WWTPs) as organic fertilizers that have the potential to contaminate water bodies and soils (Figure 1). Treatment by anaerobic digestion and/or composting can significantly reduce chemical contaminants, ARB, and ARGs (Gupta et al., 2021; Topp et al., 2018). Little doubt exists that chemicals select ARB and/or ARGs in the gut or in the environment where chemical concentrations are very high (Graham et al., 2011; Larsson et al., 2007), but what happens in environments with comparatively lower concentrations is unclear, which impairs the development of optimal barriers to address each scenario. The implementation of practices that reduce the use of antibiotics in animal production, and the deployment of improved diagnostics, along with the use of vaccines or probiotics, has shown promising results and should be encouraged worldwide (Cabello et al., 2016).

Man-made solutions

Two main strategies can be implemented to reduce environmental emissions of antibiotics. The first is through actions such as the reduction of pollution sources by reducing the use of antibiotics in human medicine and agriculture, or even developing and applying safer chemicals, taking advantage of solutions provided by green and sustainable chemistry that can be implemented (Kümmerer et al., 2019). Although pollution prevention at source can help to decrease the amounts of chemicals released in the environment, their use is not foreseen to end in the short term. Hence, the second strategy

TABLE 4: Semiquantitation of detected pesticides by nontarget analysis in wastewater effluent, surface water grab samples and polar organic chemical integrative samplers

	Fall effluent (ng/L)			Surface water grab samples (ng/L)			POCIS samplers (ng/kg POCIS)	
Compound	HR2	HR7	HR8	Reference site: Flax Pond	Hudson River: open river	Hudson River: marsh	Reference site: Flax Pond	Hudson River: marsh
Atrazine	<1	<1	<1	N.D.	N.D.	N.D.	3.6	27.3
Atrazine desethyl	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.3	4.9
Bensulide oxon	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.6
Benzoguanamine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.4	3.3
Carbendazim	11.3	6.2	10.3	N.D.	N.D.	N.D.	5.4	37.3
Cybutryne	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	<1
Diethyltoluamide (DEET)	22.4	<1	1.9	8.8	30	9.5	76.4	192
Dimethenamid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.3
Diphenamid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	N.D.
Diuron	7.8	1.3	5	N.D.	N.D.	N.D.	N.D.	N.D.
Imazapyr	2.3	16.4	0.7	N.D.	N.D.	N.D.	N.D.	N.D.
Imidacloprid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	33
Imidacloprid, desnitro	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	6.9
Isonoruron	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	<1
Metolachlor	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	9.6
Picaridin	2	<1	3.7	N.D.	N.D.	N.D.	N.D.	N.D.
Prometryn	<1	<1	<1	N.D.	N.D.	N.D.	N.D.	N.D.
Simazine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	1.9
Spiromesifen enol	<1	<1	4.4	N.D.	N.D.	N.D.	<1	<1
Triadimefon	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	<1
Trifloxystrobin acid (E,E)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<1	<1

Semiquantitation was performed using external calibration curves. Concentrations for water samples are shown in ng/L. Concentrations for POCIS samplers are shown in ng/kg POCIS.
HR = wastewater treatment plant effluent samples discharged to the Hudson River; N.D. = nondetect; POCIS = polar organic chemical integrative sampler.

involves reduction of the contamination load by remediation measures.

Domestic wastewater, including hospital effluents, are important niches where complex mixtures of chemical contaminants are mixed with human-derived bacteria, which is probably a major source of environmental antibiotic resistance (Karkman et al., 2019). Hence, WWTPs are among the primary barriers in preventing chemical and biological contaminants from reaching natural ecosystems. Standard wastewater treatment, which often involves environmental microbiota in conventional activated sludge (the most standard practice in conventional WWTPs) processes, reduces the organic load, destroys some chemical contaminants, and stimulates a native microbiome that outcompetes fecal-derived pathogens. This biological process removes significant numbers of ARGs and ARB, typically 1.5 to up to 3.0 log-units/volume. However, potentially concerning ARGs often remain after treatment (Graham et al., 2018; Manaia, 2022; Marano et al., 2021). Also, a significant proportion of micropollutants such as PPCPs (including antibiotics) are not readily eliminated in conventional activated sludge systems (Angeles et al., 2020). Therefore, treated municipal wastewater effluents can cause environmental contamination when discharged to aquatic bodies (Brunelle et al., 2022; Graham et al., 2018; Manaia, 2022), or when used for irrigation (Becerra-Castro et al., 2015).

Although more important at local scales, emissions from antibiotic manufacturing facilities can have major impacts on antibiotic resistance (Larsson et al., 2007), and there is a growing consensus that managing such discharges at the source is important and urgently needed (Milaković et al., 2019; Topp et al., 2018). Currently, many manufacturing facilities, especially in low-to-medium income countries, suffer from illegal dumping and/or insufficient and poorly considered effluent treatment. For example, the use of biological processes without effective biosolids separation is very ill suited to treat manufacturing wastes because the high chemical concentrations expected from such sources may select ARB and ARGs (Marathe et al., 2013) or promote resistance development after effluent release (González-Plaza et al., 2019).

The issue of "suitable" waste treatment at antibiotic production facilities represents a more general problem of identifying appropriate wastewater treatment options to reduce ARGs, ARB, and chemical emissions to the environment. This problem is made more difficult by the fact that the most accessible treatment options for reducing ARGs and ARB emissions in any scenario depend on local civil infrastructure, which differs dramatically between low-to-medium income and high-income country contexts (Graham et al., 2018). The WHO has recommended global implementation of "safe sanitation" as an immediate goal, and progression to secondary wastewater treatment when infrastructure and resources are more amenable (WHO et al., 2022). However, this does not resolve the problem of simultaneously reducing antibiotics, ARB, and ARGs because many technologies require infrastructure that is too costly or unfeasible for many places, such as for densely populated unplanned urban settlements in low-to-medium income countries (WHO et al., 2022). This issue is beyond the

scope of our review but must be borne in mind when one is considering treatment options.

Well-operated biological WWTPs with effective biosolids management are critical for reducing ARGs, ARB, and some chemical residue emissions. The key, however, is "effective" biosolids separation, which is essential for removing ARGs and ARB from the liquid phase and, in turn, the effluent (Quintela-Baluja et al., 2019). However, even with well-managed biosolids separation, water-soluble chemicals are not removed, and are released to the environment in the effluent. If chemicals and ARB and/or ARG removal is the goal, physicochemical treatment processes based on filtration, adsorbents, ozone, advanced oxidation processes, and other advanced procedures, such as solar oxidation, and electro- and sonochemistry can also be applied (Rizzo et al., 2020; Rodriguez-Mozaz et al., 2020).

Although specific wastewater disinfection is not mandated in WWTPs in all countries, the most common options include chlorination, UV irradiation, or ozonation. However, there is debate about the cost-effectiveness of these processes. They can, in addition, reduce antibiotic resistance (typically by 0–1 log-units/volume in full-scale systems), but can have unintended effects such as the release of cell-free DNA harboring ARGs, which eventually may be horizontally transferred to native bacteria (Manaia, 2022; Rizzo et al., 2020; Yuan et al., 2019). Furthermore, "advanced" treatment methods can have high energy demands, use hazardous chemicals. or, for chemical contaminants, produce unwanted transformation products of often unknown chemical structure, toxicity, and fate (Kümmerer et al., 2019). Conversely, for ARB and ARG removal, major limitations of advanced methods include the disturbance of autochthonous microbial communities, promoting the opportunity for ARB regrowth, the release of extracellular ARGs with unknown effects, and the demand for high doses of disinfection agent (e.g., radiation, ozone) versus exposure time to achieve effective ARG removal (Hong et al., 2018).

Finding a treatment solution efficient for the removal of both chemical contaminants and ARB and/or ARGs is challenging and may require customized solutions, for example, upstream source treatment for critical sources such as hospitals (Verlicchi, 2021); decentralized WWTPs where infrastructure is lacking or fragmented (Graham et al., 2018); and low-energy-consuming processes that employ no or passive aeration in the biological treatment step, for example, anaerobic membrane bioreactors (Maaz et al., 2019). Also, combined treatment systems, with sequential removal of chemical and biological contaminants, may be suitable options to treat effluents with complex matrices of contaminants (Rizzo et al., 2020).

With a little help from nature

Natural ecosystems offer a broad range of ecosystem services with the potential for attenuating the impacts of chemical environmental contaminants, for example, through biotransformation, photolysis, sorption, or volatilization. However, unintended and unpredictable effects may rule the natural attenuation processes, due to 1) high variability in the behavior of contaminants based on their different physicochemical properties and environmental conditions (Acuña et al., 2015); 2) transformation products generated through degradation of chemical compounds, which can sometimes be as persistant or toxic as the parent compounds (Kümmerer et al., 2019); and 3) accumulation and adsorption to sediments, from which they can be remobilized (Crawford et al., 2022).

For antibiotic resistance development and transmissionn, natural attenuation can be efficient if the receiving microbiome is strong and resilient against invasive microorganisms. The capacity of invasive bacteria, including ubiquitous ARB, to propagate in nature is strongly influenced by the microbial diversity of the receiving environment (Ribeirinho-Soares et al., 2022; van Elsas et al., 2012). Indeed, the capacity of a microbial community to retain (resistance) or restore (resilience) its structure on invasion or after a sudden alteration of the environmental conditions has been suggested to be more effective in balanced and taxonomically and functionally rich and diverse communities (Van Bruggen et al., 2019). (Waste)waters with very low levels of native microbiota due to harsh disinfection processes that were stored (up to 7 days) displayed unbalanced communities dominated by Beta- and Gammaproteobacteria classes to which belong bacterial genera often involved in antibiotic resistance transmission (Alexander et al., 2016; Moreira et al., 2021).

Low-cost ecology-based processes, such as microbiome engineering, have the potential to promote the biodegradation of chemical contaminants and to prevent the proliferation of hazardous microorganisms, including multidrug-resistant bacteria (Albright et al., 2021; Ribeirinho-Soares et al., 2022). In specific situations, ecology-based processes may be a solution to treat wastewaters. However, adequate risk assessment studies are needed prior to the implementation of such processes. In addition, it must be recognized that the natural environment may have a limited capacity to tackle the enormous amounts of chemical and biological contaminants entering the aquatic ecosystem as a consequence of anthropogenic activities. Man-made barriers are thus compulsory to counteract or at least reduce the impacts of these discharges.

The increasing world population, together with climate change, is exacerbating the need for water in sufficient quantity and quality, and reuse of treated wastewater is being fostered but also endangered by pollution (Kümmerer et al., 2019; Medlicott et al., 2020). The uptake of ARGs by crops irrigated with treated wastewater has been suggested (Cerqueira et al., 2019). However, the reuse of water for irrigation seems to be unavoidable in some world regions. The fact is that with intended or unintended water reuse, wastewater represents one of the interconnected human, animal, and environmental habitats that can contribute to the emergence, evolution, and spread of antibiotic resistance in a One-Health continuum (Hernando-Amado et al., 2019). Therefore, in terms of wastewater treatment and water reuse demand for the development and implementation of barriers to chemical and biological pollution infiltration into the environment, it is important to ensure water safety, particularly when water is intended for irrigation or human consumption (Textbox 3).

TEXTBOX 3: Human health risks and environmental antibiotic resistance

The threats posed to human health can be considered as two separate but interlinked processes: the long-term evolution of antibiotic resistance mechanisms in the environment, leading to emergence of resistance in human pathogens through mutations and horizontal gene transfer, and the more acute risk of existing ARB transmission from the environment to humans. Assessing human health risks associated with environmental antibiotic resistance is complicated by the ubiquity of microorganisms, the mobility of ARGs between bacterial taxa, microbial transmission across environmental compartments, and individual people's behaviors and underlying health.

ENVIRONMENTAL ANTIBIOTIC RESISTANCE AND THE RISK IT POSES TO HUMAN HEALTH

Once present in natural environments, ARGs may spread throughout distinct environmental compartments and reach humans and animals (Figure 1). Human exposure to environmental ARB has the potential to result in asymptomatic colonization (Leonard et al., 2018), or treatment-resistant infections (Larramendy et al., 2020). Much research has been conducted to characterize and quantify the scale of environmental antibiotic resistance, but less evidence exists on the risk of exposure, and of ARB and/or ARG transmission from the environment to humans. A recent systematic map identified 39 studies providing empirical evidence of health outcomes in humans associated with exposure to antibiotic resistance in natural environments, with the greatest research effort focusing on colonization associated with exposure to aquatic environments (Stanton et al., 2022).

Aside from conducting epidemiological studies to directly quantify the risks of colonization or infection associated with environmental exposures, source attribution models and risk assessment approaches are providing insights into the most important sources and types of antibiotic resistance. Accurate source-tracking in the environment has been confounded by the complex environmental conditions and limited accuracy and/or sensitivity of traditional methods such as tests based on indicator bacteria or marker genes. However, global wastewater monitoring and associated resistomes have clearly shown how antibiotic-resistant levels in the human gut microbiome differ around the world (Hendriksen et al., 2019). Inadequate local sanitation infrastructure, including lack of well-maintained WWTPs, public healthcare spending, education, and political factors significantly explain antibiotic resistance in local populations (Collignon et al., 2018). It is still unclear whether different prevalence and patterns of antibiotic resistance around the world can be associated with distinct levels of risk for human populations. Recently, global-scale public environmental metagenomic data sets supported by machine-learning were suggested as useful approaches for developing source-tracking and quantitative models (Li et al., 2018), which may lead to customized risk assessment frameworks in the future.

Human exposure within the One-Health continuum

Although the environmental origin of a number of ARGs in human pathogens is not in doubt, the relative contribution of anthropogenically impacted environments on the global scope of antibiotic resistance is still difficult to quantify. This is mainly due to lack of data regarding the persistence of resistant pathogens in the environment and the scope of antibiotic resistance transfer between clinical and environmental bacteria. Recently, Leonard et al. (2022) reviewed the potential exposure of people from fecal-contaminated recreational waters and concluded that the complex interactions among humans, animals, and the environment makes the health implications of environmental antibiotic resistance difficult to quantify. This same conclusion can be expanded to other types of environment. Although some authors may consider that data showing clear links between anthropogenic actions and environmental contamination are still insufficient to drive action, the high probability of transmission across multiple One-Health paths cannot be ignored. The most pressing research need is to devise means of robust source attribution, linking human contamination with any source in the One-Health continuum. The WWTP emissions (treated wastewater reused for irrigation or organic fertilizers used as a valued source of nutrients for crop production) are examples of triggers for potential antibiotic resistance exposure pathways to humans (European Food Safety Authority Panel on Biological Hazards et al., 2021; Marti et al., 2013). Urban environments with inadequate or poorly maintained wastewater treatment infrastructures, which may originate widespread waterborne contamination with emerging ARGs, as is the case with New Delhi metallo beta-lactamase-1 (NDM-1; Walsh et al., 2011), are good examples of how wastewater may be an indirect source of ARGs to humans.

Overall, fecal waste streams carrying enteric bacteria of human or animal origin will increase the abundance of ARB in terrestrial or aquatic reservoirs. In an earlier study, bathers who were regularly exposed to water impacted by wastewater had a three-fold higher probability of carrying extended spectrum β-lactamase–producing (ESBL)-*E. coli* and a four-fold higher probability of carrying $bla_{CTX\text{-}M}$-bearing *E. coli* than were non-bathers (Leonard et al., 2018). Although the source of the wastewater (e.g., WWTPs or combined sewage overflows) was unclear in that study, the results did show that wastewater exposures are associated with human antibiotic resistance carriage.

A variety of modeling approaches deployed in middle (ESBL-producing bacteria; Thailand) and higher (ESBL-producing *E. coli*

and plasmid-mediated AmpC-producing *E. coli*; The Netherlands) income settings have concluded that environmental transmission of ESBL-producing bacteria to humans occurs far less frequently than human–human transmission (Booton et al., 2021; Mughini-Gras et al., 2019). However, Mughini-Gras et al. (2019) found that the basic reproduction number (R0) for intracommunity transmission of these bacteria was below 1, suggesting that other transmission modes must occur. They concluded that the rate of human–human transmission was likely insufficient to maintain ESBL-*E. coli* and pAmpC-*E. coli* in the open community, indicating that there is ARB transmission to and from nonhuman sources. Such evidence is important for identifying targets for mitigation that will have the greatest impact for reducing antibiotic resistance in the community and improving public health. These models use comparative genomic approaches to infer transmission events but are likely to classify

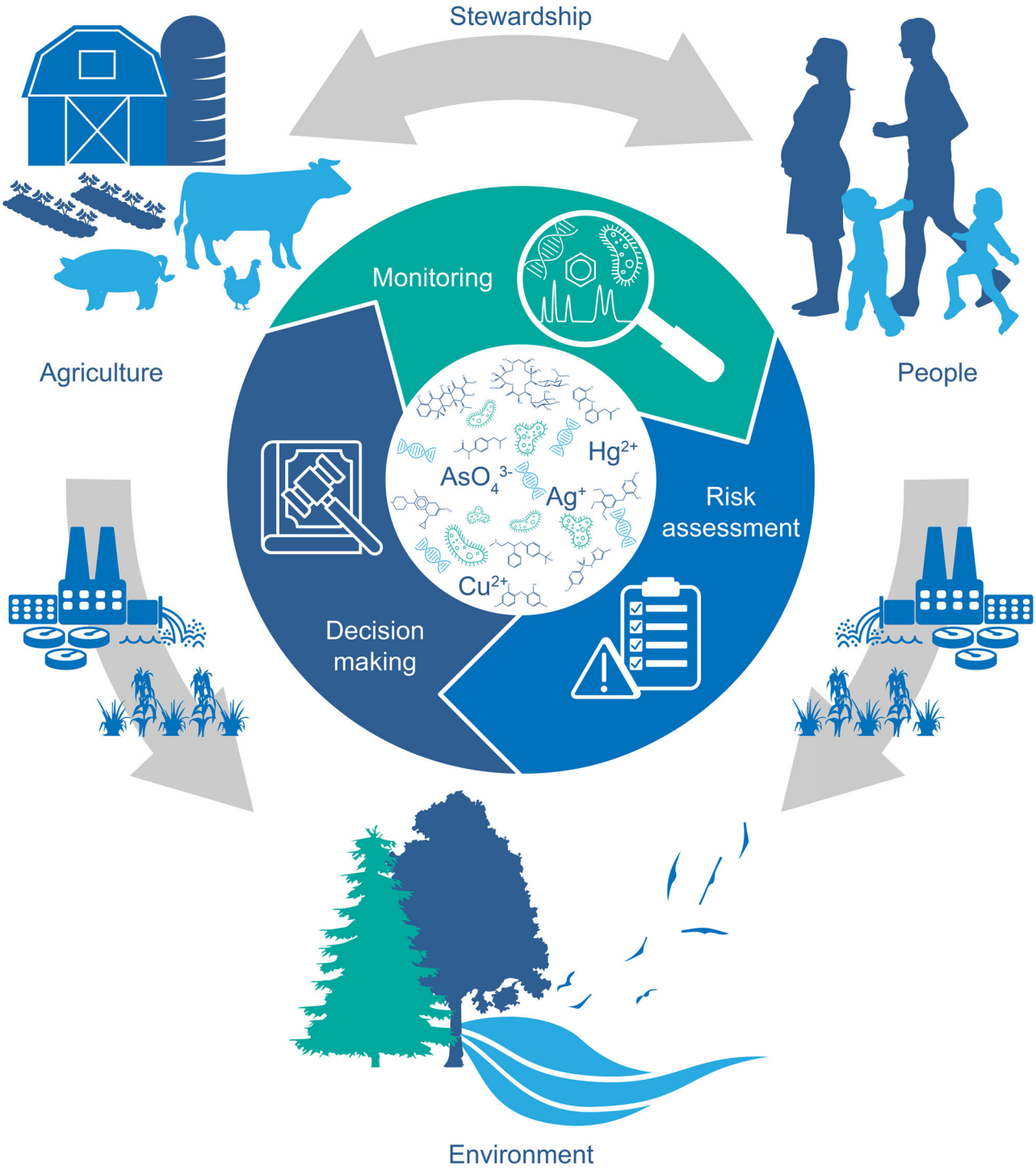

FIGURE 2: Priorities for future research and action, embracing the One-Health continuum at the global scale and the involvement of the scientific community, as well as different stakeholders, from the citizen to the decision-making entities.

wastewater-borne bacteria as human-to-human transmission when in fact these pathogens are transmitted via the environment, potentially underestimating the role the environment plays in transmission.

Another path of transmission is exemplified by humans working in the primary production, transportation, or processing environments of farm animals. In an epidemiological survey focused on livestock-associated methicillin-resistant *Staphylococcus aureus*, 31% of the 3657 human methicillin-resistant *S. aureus* cases in Denmark were due to the clonal complex 398 (LA- methicillin-resistant *S. aureus* CC398). Eighty-nine percent of these were in patients who had had previous contact with livestock production (Korsgaard et al., 2020), suggesting the dynamic movement of those pathogens between humans and animal or environmental sources.

Human health risks: From inference to evidence

Antibiotic resistance risk to human health varies according to multiple factors, including host pathogenicity, genetic context, and likelihood of transfer to human pathogens. Among the plethora of ARGs that can be observed in the environment, most are intrinsic, with natural occurrence in the respective hosts, nonmobile, and often associated with nonresistance biological roles (Alcock et al., 2020). Even though the occurrence of ARGs in the environment may be an indication of contamination, human health risks should be assessed based on the identification of high-risk ARGs (Martínez et al., 2015). These priority targets for mitigation should not be geographically restricted but should rather take on a global perspective. To identify priority ARGs, it is necessary to establish a risk ranking system, although its implementation may be limited by the availability of clinical and experimental data (Huijbers et al., 2019; Martinez et al., 2015). The existing and ever-increasing global data sets of bacterial genomes and environmental metagenomes may lessen the dependence on clinical and experimental data. Bacterial genomes data have the potential of providing a great opportunity to perform comprehensive antibiotic resistance risk assessment, because the genetic context, including host and mobility, can be resolved by genomic analysis.

Many complexities remain to be unravelled, but current advances are addressing these issues. Recently, an omics-based framework (Zhang et al., 2021) was developed to evaluate ARG risk considering human-associated enrichment, gene mobility, and host pathogenicity. This framework identified 73 "current-threat" ARG families, including 35 proposed as high risk by the WHO (2019; Zhang et al., 2021), and confirmed that "future threatening" ARGs were significantly enriched among those recently transferred into pathogens. In the future, the omics-based framework has the potential to further improve antibiotic resistance risk assessment, especially coupled with computational methods like hidden Markov models, environmental exposure studies, and experimental approaches like phenotypic characterization and functional metagenomics screens.

CONCLUSIONS

Antibiotics are plausible antibiotic resistance selectors, especially in the gut of treated individuals and animals. In the environment, these compounds normally occur at very low concentrations, which have been shown to select for antibiotic resistance in laboratory experiments. Although it is true that the low concentrations of antibiotics detected in the environment cannot be correlated with the load of antibiotic resistance, they are often present with other anthropogenic pollutants, such as PPCPs. Oxidative stress and other cellular challenges posed by nonantibiotic compounds are observed to disturb microbial communities and stimulate the acquisition of ARGs through horizontal gene transfer. This evidence suggests that antibiotic resistance drivers are probably multifactorial, and that non-antibiotic environmental contaminants may be also part of the process. Human activities (personal care, healthcare, household, agriculture, and animal farming) are the major sources of both ARB and ARGs and of a myriad of chemical contaminants. Wastewater treatment systems represent important man-made barriers capable of minimizing the spread of both types of contaminants. However, many parts of the world lack this barrier and even where present, they are still associated with environmental dissemination of ARGs and ARB and chemical contaminants. Expanding treatment should be a major global goal for reducing antibiotic resistance, beyond just using fewer antibiotics. However, insufficient efficiency of these barriers can be complemented by natural systems, such as microbiome modulation, in which the native microbiota can contribute to attenuate pollution through biodegradation or transformation of chemicals and competition or predation of bacteria. Future research priorities include the concentration of efforts to better understand the drivers, chemical contaminants, particles (e.g., microplastics, nanomaterials), microbiome permissiveness, temperature, or other factors, and above all, how the interaction among them, may influence the development, transmission, and spread of antibiotic resistance.

Despite the numerous reports demonstrating the wide occurrence of clinically relevant ARB and ARGs in the environment, it has been difficult so far to find clear evidence of direct transmission to humans. Nonetheless, it is recognized that antibiotic resistance moves across the One-Health continuum, where multiple human exposure opportunities are created. Future research priorities include the development of adequate exposure models and the implementation of sensitive resistance detection systems combined with reliable risk ranking tools contributing to the development of integrated risk assessment frameworks. The more the environment is polluted with ARB, ARGs, and compounds that can select, coselect, or maintain ARB and ARG numbers, the more numerous the opportunities will be for human (or animal) exposure, and subsequent infection or colonization from environmental reservoirs. Given the current and future threats posed by

antimicrobial resistance to human health, the global economy, and food security, changes in environmental pollution policy are needed now. Ongoing and future research can shape and inform these changes as they are implemented.

Summing up (Figure 2), a major future research priority should aim at devising strategies and technological solutions that holistically deal with environmental contamination, which will reduce the transmission and spread of antibiotic resistance and decrease human health risks. Although this goal may seem difficult to achieve, advances in high-throughput analytical methods, multi-omics approaches, and machine-learning tools may contribute to generate holistic insights and actions to minimize pollution. However, improving waste management on global scales might have a more immediate impact.

Four major priorities for future research:

1) Elucidate the role of interactions among complex mixtures of chemical contaminants and microbiota in natural and man-made environments that contribute to the emergence or dissemination of ARGs, with recognized or potential clinical relevance;
2) Implement sensitive and accurate quantification methods for monitoring mixtures of chemical and biological contaminants, at critical control points;
3) Improve the capacity of man-made and natural barriers, to alleviate the load of antibiotic resistance and chemical contamination, mainly from wastewaters, sludges, or manure prior to the discharge or deposit in the environment;
4) Clarify major routes of exposure (e.g., surface water, water reuse for agricultural irrigation, aquaculture), infectious doses, and risks to humans and animals posed by environmental antibiotic resistance (Figure 2).

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