OPINION

Tackling antibiotic resistance: the environmental framework

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Abstract | Antibiotic resistance is a threat to human and animal health worldwide, and key measures are required to reduce the risks posed by antibiotic resistance genes that occur in the environment. These measures include the identification of critical points of control, the development of reliable surveillance and risk assessment procedures, and the implementation of technological solutions that can prevent environmental contamination with antibiotic resistant bacteria and genes. In this Opinion article, we discuss the main knowledge gaps, the future research needs and the policy and management options that should be prioritized to tackle antibiotic resistance in the environment.

The global spread of antibiotic resistance genes (ARGs) and their acquisition by clinically relevant microorganisms is associated with the increased hospitalization and mortality rates of patients that are infected with such microorganisms, which constitutes a serious problem for the health and welfare of both humans and animals[1]. The effect of clinically relevant ARGs and antibiotic resistant bacteria (ARB) that are released from anthropogenic sources, together with the excessive use of antibiotics in both human and veterinary settings, is currently considered to be a serious environmental problem[1–5]. However, current risk assessment models are inadequate to evaluate the effect of antibiotics and ARGs on resistance emergence and selection, especially in non-clinical environments.

In contrast to many chemical contaminants — the concentration of which typically diminishes owing to degradation, dilution or sorption — bacterial contaminants (and their ARGs, which are present both within bacterial genomes and in free DNA) are capable of persisting and even spreading in the environment. The ARGs carried by these bacterial contaminants can multiply in their hosts, be passed on to other bacterial populations and be subject to further evolution. As such, ARB that occur in the environment represent potentially serious risks for human health.

The increased dissemination of ARB in the environment is probably caused by three principle mechanisms, which can occur in combination: horizontal gene transfer of ARGs; genetic mutation and recombination (which can be favoured by the existence of hypermutator bacterial strains); and the proliferation of ARB owing to selective pressures that are imposed by antimicrobial compounds or other contaminants, such as biocides or heavy metals[6–10]. As bacterial communities are shaped by a complex array of evolutionary, ecological and environmental factors, it is difficult to predict the fate of ARGs and ARB that are released into the environment, or to obtain a clear understanding of the evolution and ecology of antibiotic resistance in this setting. For example, although the excessive use of antibiotics may select for resistant populations in the environment, other biotic and abiotic factors (such as physicochemical conditions, environmental contaminants, induction of stress responses, bacterial adaptation and phenotypic heterogeneity) have the potential to enhance the effect of selective pressures and promote bacterial evolution towards antibiotic resistance. Conversely, there is still a poor understanding of the environmental factors that may alleviate the spread of antibiotic resistance.

Antibiotic resistance hotspots are found not only in medical settings but also in environmental compartments that are subjected to anthropogenic pressure, such as municipal wastewater systems, pharmaceutical manufacturing effluents, aquaculture facilities and animal husbandry facilities. These sites are characterized by extremely high bacterial loads coupled with subtherapeutic concentrations of antibiotics, and they contribute to the discharge of ARB and ARGs into the environment. At present, it is not clear to what extent environmental ARB and ARGs promote the acquisition and spread of antibiotic resistance among clinically relevant bacteria, or whether ARGs that are acquired by both clinically relevant bacteria and strictly environmental bacteria originate from the same reservoirs[11–13]. However, for these issues to be properly addressed, global efforts are required to characterize and quantify antibiotic resistance in the environment. In particular, it is necessary to create an adequate support for advanced biological risk assessment evaluations, which are needed to determine how contaminated environments affect the proliferation of antibiotic resistance. In parallel, the implementation of technological solutions that can reduce the contamination of natural ecosystems by clinically relevant and potentially evolving ARB and ARGs is also a priority.

These topics were the focus of the European COST (Cooperation in Science and Technology) Action DARE (Detecting Evolutionary Hotspots of Antibiotic Resistance in Europe, TD 0803), which was launched in 2009 and completed in 2013. This interdisciplinary project involved 20 European countries and 123 scientists with a wide range of backgrounds (for example, engineers, microbiologists, chemists, veterinarians and physicians, working at

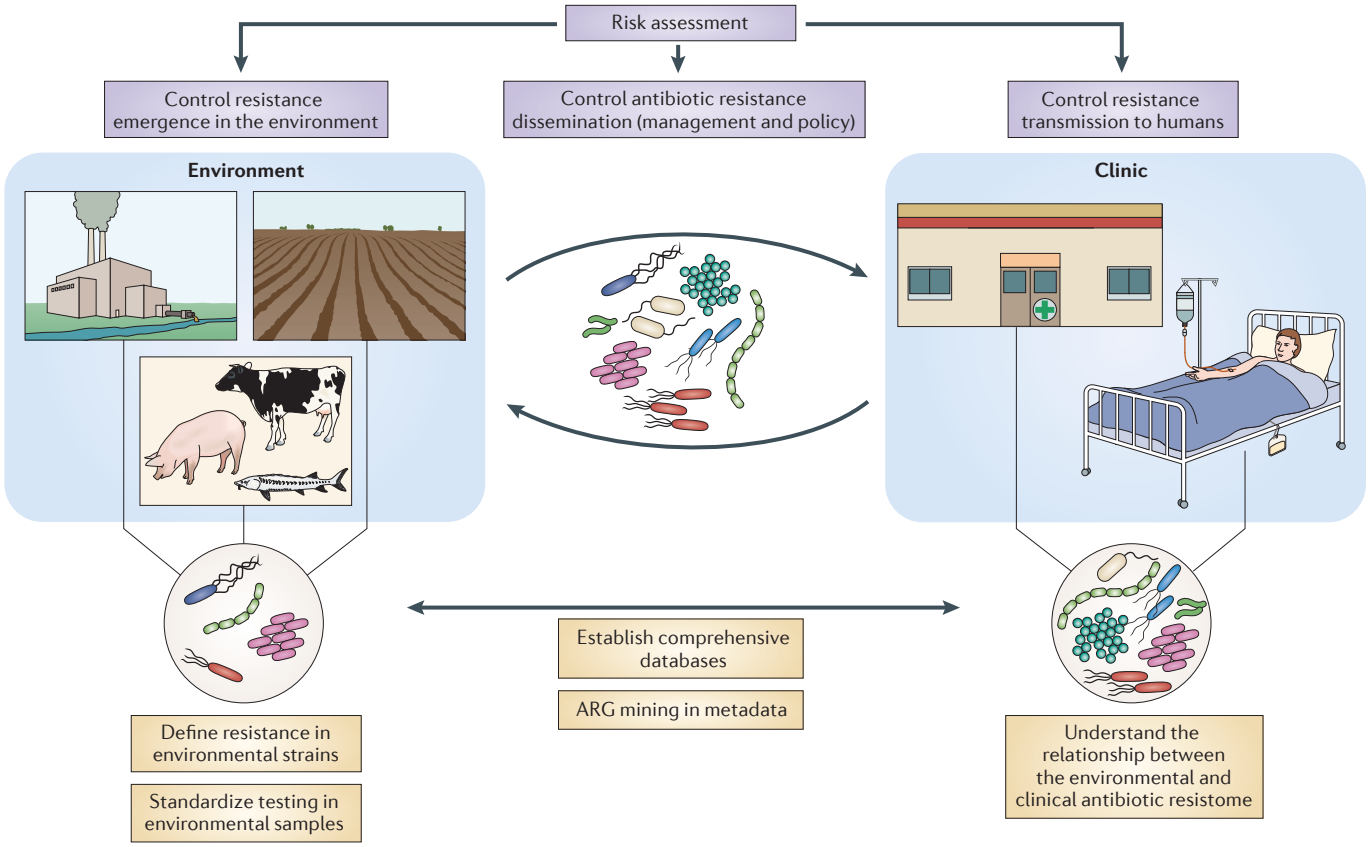

Figure 1 | **Minimizing the spread of antibiotic resistance in the environment.** The figure summarizes the current goals (purple boxes) in trying to minimize the emergence and spread of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) in the environment and their transmission into the clinic. The current needs and limitations that must be resolved to achieve these goals are also shown (yellow boxes). To evaluate the spread of antibiotic resistance in the environment, and the risk of transmission to humans, it is necessary to define what constitutes resistance in environmental bacterial strains and to standardize testing in environmental samples. This improvement in the definition and testing of resistance should contribute to the establishment of more comprehensive databases that combine data from both environmental and clinical settings. These databases would contribute to the evaluation of the relationship between the antibiotic resistomes in both settings and facilitate the mining of ARGs in metadata. These strategies would improve the assessment of the risk of dissemination of ARB and ARGs in the environment and their transmission to humans, and they would potentiate the development of control strategies (management and policy) aimed at preventing the dissemination of antibiotic resistance.

universities, research institutes, national health agencies and national veterinary agencies). In this Opinion article, we present our consensus on the main knowledge gaps, future research needs, policy options and management options that should be prioritized when tackling antibiotic resistance in the environment (FIG. 1). A holistic view of antibiotic resistance evolution is proposed, which considers the emergence and dynamics of ARB and ARGs in relevant environmental compartments and includes the identification of both conditions that can enhance its spread and relevant points of control.

Standardization of resistance testing

Over the past two decades, important advances have been made regarding the international implementation of standardized methods to monitor antibiotic resistance in clinical isolates. These advances were essential in enabling the comparison of resistance prevalence in different geographical regions to assess possible relationships between antibiotic resistance and antibiotic consumption, and to acquire a temporal perspective on resistance dynamics[1]. Despite the ever-increasing evidence that the evolution and spread of antibiotic resistance in the environment contributes to the occurrence of antibiotic resistance in clinical or urban settings[2–9], standardized methods that are directly applicable to environmental samples (which enable reliable comparisons with clinical data) have never been developed. Therefore, microbiologists and environmental scientists worldwide have adapted clinical criteria — usually based on pre-established breakpoints, which offer an objective classification of an organism as either resistant or susceptible to a given antibiotic[10] — to define and examine antibiotic resistance in water, sludge, sediment, plant, manure and soil samples[14–16]. However, the conditions and criteria for bacterial isolation, and the number of isolates that are necessary to obtain a representative set of species (or even the prevailing species), are completely different in environmental samples compared to those in clinical samples. Furthermore, most environmental bacteria are not recovered in culture-dependent surveys. Last, the definitions of resistance used for clinical isolates, which are mainly based on the likelihood of therapeutic failure of human or animal bacterial infections, may not apply to environmental bacteria[15,16]. These shortcomings have motivated the development of methods that can give a more comprehensive overview of the prevalence of resistance in environmental samples.

One approach that has been used to evaluate the status of antibiotic resistance

in the environment involves the calculation of resistance percentage, which is based on the ratio between the number of bacteria that are able to grow on culture media that are supplemented with antibiotics at doses close to the minimal inhibitory concentration (MIC) and the number of bacteria that grow on antibiotic-free media[16]. Culture-independent approaches, which are mainly based on information about ARGs that was previously acquired from the study of clinical isolates, have also been successfully developed[17–21]. In particular, quantitative PCR (qPCR) can give an approximation of the prevalence of known ARGs in environmental samples, which can be a good estimation of the level of contamination by ARGs, although careful standardization of gene copy numbers is needed. However, as different methodologies are regularly used, the results obtained by these approaches cannot be compared with those commonly used in surveillance reports on human medicine and veterinary medicine[1,22]. Therefore, harmonized guidelines are needed regarding the number of isolates and diversity of species or strains to be tested; the cultivation conditions or the DNA extraction methods; and the targeted resistance phenotypes and genotypes or primer sets. Such guidelines would enable direct comparisons between different environmental compartments, thus establishing bridges with clinical data.

Standardization of resistance testing should further focus on bacterial indicators that are already in use and also on a subset of resistance determinants, preferably with both analysed concurrently. Primary bacterial indicators should be members of the class Gammaproteobacteria or the phylum Firmicutes, as these are the most frequent carriers of acquired ARGs (BOX 1). In particular, we propose *Escherichia coli* and faecal enterococci — which are currently used to monitor microbiological water quality and are well characterized in terms of acquired antibiotic resistance — as indicator organisms[16]. In addition, *Pseudomonas aeruginosa*, which is also used as an indicator of water quality, and *Aeromonas* spp., which are typical water inhabitants, should be used for the examination of environmental samples in which faecal contamination is not expected. *Klebsiella* spp., in particular *Klebsiella pneumoniae,* are also possible indicators to consider, as members of this group are present in both the environment and the animal gut and have frequently been found to be pioneers in the emergence of antibiotic resistance[23]. Although other indicator bacteria would be eligible, the bacteria mentioned above have the advantage of being ubiquitous as well as being important carriers of antibiotic resistance and being responsible for the transfer of ARGs between different environmental compartments[13,23–26].

For the selection of resistance determinants, criteria such as the clinical relevance, the prevalence in the environment, the association with mobile genetic elements (MGEs) and/or the potential to be acquired by any mode of horizontal gene transfer (such as conjugation, transformation or transduction) are important. Possible candidate genes, frequently occurring in environmental settings that are subjected to human activities[9,16,27,28], are shown in BOX 1.

These recommendations should not be regarded as attempts to narrow the focus of researchers; however, there is an urgent need for standardization of a number of core parameters to improve the comparability between studies worldwide. This is a prerequisite for obtaining a global perspective of the environmental antibiotic resistome irrespective of the geographical region, the time frame or the environmental compartment being analysed.

The importance of global databases

The current state of knowledge is insufficient to assess the distribution and abundance of ARB and ARGs in the environment at national, regional or global levels, which is a major limitation in determining the current risk of transmission of antibiotic resistance from the environment to human-associated bacteria. There are three main reasons for this limitation: the existing data are not comparable, as there are no guidelines for data collection; there is no formal system of data collation and curation for publication; and surveillance in environmental compartments or ecosystems (such as soil and water) has not been encouraged. Therefore, most of the available data come from sporadic research studies rather than long-term monitoring efforts.

Current limitations. Phenotypic resistance is often interpreted based on clinical standards and recommended breakpoints, for example, from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI). However, the use of clinical breakpoints to assess the antibiotic susceptibility of environmental bacteria is inadequate, as clinical breakpoints are based on parameters that are only relevant for therapeutic success. A more reliable alternative for the interpretation of the antibiotic resistance of environmental bacteria may be the epidemiological cut-off (ECOFF) value developed by EUCAST, which, in a given

Box 1 | **Bacterial groups and genetic determinants**

The following bacterial groups and genetic determinants have been suggested as possible indicators to assess the antibiotic resistance status in environmental settings.

Bacterial groups

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Aeromonas* spp.
- *Pseudomonas aeruginosa*
- *Enterococcus faecalis*
- *Enterococcus faecium*

Genetic determinants (and the proteins they encode)

- *intI1* (integrase gene of class 1 integrons, a genetic platform for ARG capture)
- *sul1* and *sul2* (sulfonamide-resistant dihydropteroate synthase)
- bla_{CTX-M} and bla_{TEM} (β-lactamases, frequently identified in *Enterobacteriaceae*)
- bla_{NDM-1} (New Delhi metallo-β-lactamase)
- bla_{VIM} (carbapenemase, frequent in clinical *Pseudomonas aeruginosa* in certain areas)
- bla_{KPC} (*Klebsiella pneumoniae* carbapenemase)
- *qnrS* (quinolone pentapeptide repeat family)
- *aac*-(6′)-*Ib-cr* (aminoglycoside acetyltransferase)
- *vanA* (vancomycin resistance operon gene)
- *mecA* (penicillin binding protein)
- *ermB* and *ermF* (rRNA adenine N-6-methyltransferase, associated with macrolide resistance)
- *tetM* (ribosomal protection protein, associated with tetracycline resistance)
- *aph* (aminoglycoside phosphotransferase)

taxonomic group, separates the populations with acquired resistance mechanisms (non-wild-type) from the wild-type populations that have no resistance. In contrast to clinical breakpoints, the ECOFF values are epidemiologically based, do not relate to the therapeutic efficiency and do not differ among different committees (for example, EUCAST and CLSI)[10]. However, current ECOFF estimations use databases in which, for a given species, the number of isolates with a clinical origin is several orders of magnitude higher than that of isolates with an environmental origin. In addition, most ECOFF studies comprise microorganisms of relevance to human health (pathogens and commensals), whereas information regarding non-pathogenic environmental species, which can be important carriers of ARGs, is scarce. Hence, it is questionable whether the current ECOFF values correctly reflect the distribution of resistant and wild-type bacteria in the environment. To improve the reliability of these data, and therefore their usefulness in the classification of bacteria of different origins, it is essential to supplement ECOFF databases with additional data from environmental species and isolates.

There are several public databases and global surveillance projects, such as the Antimicrobial Resistance Global Report on Surveillance from the World Health Organization (WHO)[1]; the European Centre for Disease Prevention and Control (ECDC)-based European Antimicrobial Resistance Interactive Database (EARS-Net), EUCAST, and the European Antimicrobial Susceptibility Surveillance in Animals (EASSA)[29] in Europe; the Surveillance Network Database (TSN) in the USA and Australia; and the Study for Monitoring Antimicrobial Resistance Trends (SMART) task force[30] in the Asia–Pacific region. In addition, there are two centralized databases on ARGs: the Comprehensive Antibiotic Resistance Database (CARD)[31] and the Antibiotic Resistance Genes Database (ARDB)[32]. However, the number of ARGs described in human and animal opportunistic pathogens in these databases is much higher than the number of ARGs described in environmental bacteria. Additionally, public databases that incorporate data from environmental bacteria or metagenomes contain genes that have been putatively annotated as ARGs based on the similarity of their nucleotide or amino acid sequences to those of previously described ARGs. However, a functional demonstration of the role of many sequences described as 'resistance genes' in specialized databases is lacking, which creates a background of potentially misleading information for researchers and clinicians. The lack of a proper definition of antibiotic resistance for environmental strains of bacteria, the numerous databases with scant information on antibiotic resistance in the environment and the lack of functional demonstrations for ARGs in environmental metagenomes are considerable limitations for the characterization of the environmental resistome and the assessment of its clinical relevance[8].

Perspectives. The consolidation of specialized databases will certainly contribute to a standardized definition of ARGs and elucidate which genes contribute to the future acquisition of resistance by human pathogens. In parallel, the continuous improvement of techniques for the cultivation of bacteria using multiple culture media and conditions (known as culturomics) is of vital importance for the identification of ARGs and for understanding the cellular mechanisms linking ARGs and resistance phenotypes in different bacteria[33]. One of the greatest challenges in creating a consolidated database lies in establishing a standardized methodology and transforming it into a routine activity of environmental-quality monitoring. However, this is necessary if we are to acquire a coherent picture of environmental resistance.

Conceptually, two types of data may be integrated in public databases: data from routine monitoring (that is, from surveillance databases), which will give insights into the distribution, prevalence, temporal trends and geographical trends; and data gathered by research studies (that is, from emergence and evolution databases), which are seeking to understand the acquisition and molecular evolution processes. As one of the most important issues concerning environmental antibiotic resistance is its possible implications in the health of humans and animals, databases focusing on environmental bacteria should also be linked to existing databases on ARB and ARGs in clinical, veterinary and food-associated products. The core entries of these integrative databases should comprise the ARB and ARGs described in BOX 1 and adopt the format of existing databases (for example, EARS-Net and REFS 31,32).

The information provided by surveillance databases would be substantially improved by the inclusion of other, systematically chosen, meta-parameters. Examples include the occurrence of heavy metals, antibiotic residue concentrations and, whenever possible, the composition of the microbial community, which would be relevant for medium- to long-term evaluations of resistance evolution[34–36]. This information could be linked to routine monitoring assays of environmental samples, particularly wastewater and surface water samples[37,38]. Whenever possible, links to existing databases on MGEs (for example, A Classification of Mobile Genetic Elements (ACLAME), Insertion Sequence finder (ISfinder) or INTEGRALL) and eco-toxicological information on molecules with antibacterial effects (for example, from the European Chemicals Agency (ECHA)) should be implemented to provide a comprehensive overview of the risk factors associated with specific antibiotic resistance (for example, selection, mutation and horizontal gene transfer).

In addition to the creation of integrative databases, additional data on ARGs in the environment are also necessary. Research on uncharacterized ARGs and associated MGEs, which are not included in the routine monitoring of environmental samples, is essential to have a thorough understanding of the environmental resistome. Whole-genome and transcriptome analyses — including those that apply next-generation sequencing (NGS) technology — and functional metagenomic studies present new possibilities for deciphering resistance in environmental compartments. Studies on the expression and function of resistance genes will be fundamental in understanding the interplay between the environmental conditions and the genomic context, and in understanding how this relationship will influence the selection of specific ARGs[39,40]. These insights will offer the possibility of assessing the effect of external conditions, such as the presence of subinhibitory concentrations of antibiotics, on the presence, expression and functionality of new and clinically-associated ARGs in the environment. Last, these strategies may unveil unforeseen hotspots of antibiotic resistance. Continuous improvements on annotation procedures, such as those recently reported[31,41–45], will not only contribute to the annotation of ARGs in metadata, but will also provide valuable information about the enzymes that are responsible for each resistance mechanism, mainly concerning new genes. The association of these data to both generic (for example, the European Molecular Biology Laboratory (EMBL) or GenBank) and metagenomic databases (for example, Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST)) would further provide access to related sequence data and metadata from gene fragments to metagenomes, transcriptomes and proteomes.

However, the most important associations to achieve would be those between data from environmental and clinical settings, as well as the systematic combination of information obtained through research and routine monitoring practices. This bridging of information will be a step forwards in elucidating the role of environmental ARGs in the emergence and evolution of clinically-relevant antibiotic resistance.

Risk assessment

Integrated risk assessment of the evolution and emergence of antibiotic resistance in the environment addresses two main issues: first, the potential of subinhibitory concentrations of antibiotics to promote the development of ARB in complex bacterial communities; and second, the capacity of resistance determinants to transfer from anthropogenic sources (such as treated wastewater, manure or others) to human commensal or pathogenic bacteria.

Potential of subinhibitory antibiotic concentrations. The *Guideline on the environmental risk assessment of medicinal products for human use*, produced by the European Medicines Agency[46], does not recognize that the emergence and proliferation of antibiotic resistance may be the most important risk associated with environmental contamination by antibiotics. Indeed, the endpoints for no-effect concentrations (NOECs; which correspond to the highest concentration at which a substance has no significant effect on the organisms exposed to it) are different from traditional environmental risk assessment, as the effects of antibiotics in promoting antibiotic resistance can go far beyond the toxicological implications. For example, even at levels that are considered safe according to the currently accepted Environmental Quality Standards[47], antibiotics can still select for ARB[48–50]. Furthermore, these subinhibitory concentrations of antibiotics and antibiotic combinations may even induce the propagation of unforeseen multidrug-resistant opportunistic pathogens[51–54]. Moreover, the effects of antibiotics may be potentiated or extended by cofactors (general stress situations and micro-contaminants, such as heavy metals and biocides), which possibly enhance the spread and evolution of antibiotic resistance[34,54–59]. Therefore, combined molecular- and culture-based methods are necessary to determine the concentrations at which resistance acquisition and selection is likely to occur in environmental compartments.

An addendum emphasizing the need to assess the risks posed by antimicrobial agents of inducing ARB selection or ARG emergence should be included in the *Guideline on the environmental risk assessment of medicinal products for human use*. Although challenging, given the scarcity of knowledge regarding the mechanisms involved at the genetic, cellular and population levels, this addendum is urgently needed. Within this addendum the gold standard of a reliable risk assessment should determine the range of concentrations at which, under defined conditions, an antibiotic can promote selection and the acquisition of resistance.

Transmission of resistance determinants from anthropogenic sources. Another important aspect of antibiotic-resistance risk assessment refers to the spread and transmission of resistance determinants from hotspots to downstream environments. Mathematical models capable of predicting the influence of potential selective pressures, or the occurrence and the evolutionary success of genetic recombination events, have proven to be promising tools in predicting the spread of antibiotic-resistance determinants[54,60,61]. As they are specifically developed for environmental niches and environment–human interfaces, these mathematical models should rely on parameters such as population size; bacterial population growth rate and survival; occurrence and frequency of horizontal gene transfer and its implications on the population fitness; and the influence of other biotic and abiotic factors[54,60–62]. Such models would allow predictions to be made regarding the dynamics of ARB hosting ARGs and the possible localization of ARGs on MGEs, thus supporting the assessment of their fate from anthropogenic sources to downstream environments. A quantitative risk-assessment framework should be developed by coupling data and analyses, such as those outlined above, with a stochastic assessment of exposure to clinically relevant bacteria in the environment. Such a model should then be used to predict the environmental conditions that are associated with the evolution of antibiotic resistance and infer the probability of antibiotic resistance determinants spreading. However, owing to the scarcity of data on the occurrence of antibiotic resistance and horizontal gene transfer in the environment, it is currently difficult to develop validated models that can be applied in the framework of environmental risk-assessment guidelines.

Management and policy options

Management and policy options aimed at preventing and controlling antibiotic resistance in the environment comprise several different aspects, including the choice of ARB and ARGs to be listed as contaminants of emerging concern; the determination of differentiated maximum admissible levels of an antibiotic, ARB or ARG; and the identification of critical points of control at which prevention and remediation measures should be implemented.

ARB and ARGs as contaminants of emerging concern. The European Water Framework Directive establishes the requirements for determining the biological and chemical quality standards of water bodies in Europe[63]. Annex I of this directive sets obligations for environmental quality standards (EQS) for priority substances and certain other pollutants, and it even identifies priority hazardous substances. The inclusion of ARB and ARGs as priority contaminants would be justified based on the results of numerous scientific studies, which show that the occurrence of antibiotic resistance increases in bodies of water (such as inland surface waters, transitional waters, coastal waters and groundwater) when they are subjected to anthropogenic impacts, such as wastewater effluents, animal manure, agricultural runoff and wildlife living in urban areas[21,34,64–67]. We suggest the inclusion of a supplement to the European Water Framework Directive for ARB hosting clinically relevant ARGs. In this context, it is important to establish differentiated guidance levels for the abundance of these biological contaminants in the environment. The application of these guidance levels may be especially important for the regulation of specific practices, such as reuse of wastewater or soil fertilization with manure.

The inclusion of ARB and ARGs in the list of contaminants of emerging concern would require clear definitions on the necessary monitoring methods. Although the environmental survival of ARGs primarily depends on the host and the type of MGEs, the estimation of the levels of ARGs seems a reliable and feasible method to monitor antibiotic resistance. However, a crucial issue that needs additional investigation is the selection of target ARGs to be monitored as indicators of resistance and the determination of safe concentrations of these genes in water. Ideally, such indicator genes should be abundant in anthropogenic sources and rare in native aquatic and terrestrial ecosystems to facilitate spatial and

temporal source tracking in the environment. ARGs that are associated with MGEs or that present the highest environmental fitness (that is, long lasting survival and the capacity to proliferate) are good candidates — for example, *sul*1 (which encodes for sulfonamide-resistant dihydropteroate synthase); bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{VIM} and $bla_{NDM\text{-}1}$ (which encode β-lactamases); *tet*M (which encodes for tetracycline resistance); and *van*A (which encodes for vancomycin resistance) (BOX 1). However, determination of the maximum acceptable levels of these genes in the environment seems, at the current state of knowledge, a challenging objective. The establishment of a comprehensive database and the use of modelling approaches would be valuable contributions to estimate such limits. Despite these challenges, this knowledge is an essential prerequisite, not only for establishing a strategy of direct action against antibiotic resistance in the environment, but also for the application of drugs and interventions directed at preventing the emergence and evolution of ARB and ARGs (eco–evo drugs)[68].

Critical control points. Environmental hotspots, where ARB are abundant or the transfer of ARGs is promoted, are critical points for resistance control. Good examples of such critical points are characterized by a high prevalence of resistance or by the occurrence of resistance determinants of emerging concern. These locations comprise habitats that are influenced by human activities, such as wastewater (that is, hospital, urban and specific industrial wastewater) and waste, and wastewater from animal husbandry and intensive food-production facilities[16,69,70]. Moreover, sites subjected to the frequent discharge of antibiotic residues have been shown to be potential hotspots for the selection, proliferation and spread of new resistance determinants to human commensal and pathogenic bacteria, and these sites should likewise be considered as critical control points[71,72].

Although some of the antimicrobials administered to animals are used exclusively in veterinary applications, most belong to the same structural families that are used in human medicine. As they share the same basic chemical molecular structures and mechanisms of action, these antibiotics are assumed to put selective pressures on human commensal and pathogenic bacteria. Large quantities of antibiotics that are administered to animals in intensive production sites are discharged, often un-metabolized, with manure and slurry when applied as fertilizer (often in a raw and unstabilized state) and thus contaminate soils as well as surface water and groundwater. At present, it is difficult to ascertain whether antibiotics reaching the environment at low concentrations exert a substantial selective pressure on ARGs or ARB[50,51]. However, there is increasing evidence showing that repeated exposure of the environment to anthropogenically generated ARGs (for example, soil manure) correlate with the emergence and proliferation of ARGs in indigenous microbiota[66,67,73,74]. However, the impact of animal production on the propagation of antibiotic resistance is demonstrated by some zoonotic species of the genera *Salmonella*, *Campylobacter*, *Listeria*, *Staphylococcus*, *Enterococcus* and *Escherichia*, which are known to exhibit high levels of acquired antibiotic resistance[29,75–78]. Although some animal production facilities implement systems that decontaminate liquid and solid wastes, such treatments are not intended to remove ARB and might even promote resistance[79,80]. The same might be true for biogas reactors, which use manure as a substrate, because the residues of these reactors are used as fertilizers on agricultural fields[81]. The confinement and treatment of effluents and (processed) manure from intensive animal production is thus a priority.

Urban, hospital and pharmaceutical industry wastewater is among the main sources of antibiotic and ARB contamination in soil and water ecosystems[16,21,82]. In the environment, these contaminants can reach water resources for drinking water production, enter the food chain or reach clinically relevant niches[9,16,17,76,82]. These effects can be potentially even more pronounced when irrigation with wastewater effluents (wastewater reuse schemes) is applied. Water reuse is already a common practice in many regions of the world owing to increased water scarcity, mainly in arid and semi-arid regions[20]. Most of the wastewater treatment plants worldwide, in particular those using mechanical and biological treatments, are primarily designed to remove organic compounds, nutrients (for example, nitrogen and phosphorous) and suspended solids. However, the currently available wastewater treatment processes have limited capability to efficiently remove organic micropollutants, including antibiotics and other antimicrobial agents[82]. Similarly, certain ARB and ARGs can survive the wastewater treatment processes with a maintenance (or even an increase) of resistance prevalence compared to the pretreatment levels[16,17,36,64]. These features require the immediate attention of the scientific community and the development and implementation of technological solutions capable of mitigating ARB and ARGs in wastewater to safe levels. Although the definition of a 'safe level' may be difficult to achieve, it is at least necessary to find an agreement on the threshold values below which the probability of significant proliferation of an ARG is severely impaired.

Technologies for the removal of micropollutants, including antibiotics, and microorganisms from wastewater are becoming increasingly available (for example, membrane filtration, activated carbon, photo-driven technologies and ozonation)[82]. However, additional research is needed to determine the effectiveness of these processes for the elimination of ARB and ARGs and to characterize the associated microbiological risks[16,82]. Recommendations of effective and economically sustainable interventions at critical points within the wastewater stream are urgently needed.

Concluding remarks

Given the public health threat posed by antibiotic resistance, the development and implementation of national and international guidelines for the biological risk assessment of the emergence and propagation of ARB in the environment is a strategic priority. The generation of reliable comparisons and evaluation of temporal trends in antibiotic resistance in the environment are currently seriously limited owing to the disparity of surveillance strategies. To address this issue, the interdisciplinary scientific community involved in the DARE Action has proposed specific priority measures.

It is necessary to improve the comparability between studies worldwide to provide the basis for a global perspective on the antibiotic resistome irrespective of geographical, temporal or environmental constraints. A formal system for the collation and curation of data for publication must be implemented, and surveillance of environmental samples must be encouraged, to comprehensively assess antibiotic resistance in environmental bacteria. Databases with multiple levels of information and metadata, supporting the understanding of antibiotic resistance dynamics on a global scale, must be established. An integrated risk-assessment platform must include the potential of subinhibitory concentrations of antibiotics to promote the development of ARB in complex environmental communities, and mathematical models are required to devise reliable risk-assessment guidelines on the spread of antibiotic resistance from

the environment to human commensal or pathogenic bacteria. A selection of sentinel ARB and ARGs should be included in the list of contaminants of emerging concern and maximum admissible levels of these contaminants should be defined, as well as the critical points of control at which prevention and remediation measures can be implemented. Other pressing management options include the reduction of antibiotic usage; the confinement and treatment of problematic reservoirs, such as effluents and manure from intensive animal production or hospital effluents; and the improvement of current wastewater treatment technologies.

Finally, it is important to acquire a more comprehensive understanding of the molecular, evolutionary and ecological mechanisms associated with the acquisition and spread of antibiotic resistance. In parallel, the implementation of effective management options, mainly seeking to impose barriers against the dissemination of resistance from well-established resistance reservoirs, is urgently needed to combat the evolution and spread of antibiotic resistance, while protecting human health and the environment.

Better control of the emergence and dissemination of ARB and ARGs does not replace the need to seek new therapeutic drugs. On the contrary, by minimising the evolution of ARB and ARGs, it also prepares the ground for new drugs to be effective for a longer period of time.

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Competing interests statement

The authors declare no competing interests.

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