



Review article



Towards monitoring of antimicrobial resistance in the environment: For what reasons, how to implement it, and what are the data needs?

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ABSTRACT

Antimicrobial resistance (AMR) is a global threat to human and animal health and well-being. To understand AMR dynamics, it is important to monitor resistant bacteria and resistance genes in all relevant settings. However, while monitoring of AMR has been implemented in clinical and veterinary settings, comprehensive monitoring of AMR in the environment is almost completely lacking. Yet, the environmental dimension of AMR is critical for understanding the dissemination routes and selection of resistant microorganisms, as well as the human health risks related to environmental AMR. Here, we outline important knowledge gaps that impede implementation of environmental AMR monitoring. These include lack of knowledge of the 'normal' background levels of environmental AMR, definition of high-risk environments for transmission, and a poor understanding of the concentrations of antibiotics and other chemical agents that promote resistance selection. Furthermore, there is a lack of methods to detect resistance genes that are not already circulating among pathogens. We conclude that these knowledge gaps need to be addressed before routine monitoring for AMR in the environment can be implemented on a large scale. Yet, AMR monitoring data bridging different sectors is needed in order to fill these

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knowledge gaps, which means that some level of national, regional and global AMR surveillance in the environment must happen even without all scientific questions answered. With the possibilities opened up by rapidly advancing technologies, it is time to fill these knowledge gaps. Doing so will allow for specific actions against environmental AMR development and spread to pathogens and thereby safeguard the health and wellbeing of humans and animals.

1. Introduction

The “silent pandemic” of antimicrobial resistance (AMR) is a major healthcare challenge and is estimated to cause more than ten million deaths every year in just a couple of decades if it cannot be controlled (Murray et al., 2022; Review on Antimicrobial Resistance, 2016). Traditionally, attention has been singularly targeted towards clinical, veterinary and food-producing animal settings, but during the last decade, the environment has been increasingly recognized as having a significant role in the development and spread of AMR (Bengtsson-Palme et al., 2018; D’Costa et al., 2011; Finley et al., 2013). In recognition of microbiological systems being interconnected and interacting with each other, the One-Health approach, which includes human-associated (both clinical and in the general population), animal-associated (veterinary) and environmental settings, is instrumental to curb future resistance development in pathogenic microorganisms (Collignon, 2013). In order to understand AMR dynamics, it is of particular importance to target the interfaces between these biological compartments.

Monitoring of AMR has been implemented in clinical (World Health Organization, 2021a; WHO Regional Office for Europe and European Centre for Disease Prevention and Control, 2021) as well as veterinary, mostly food-producing animal, settings (European Food Safety Authority et al., 2019). In contrast, comprehensive and comparative monitoring of AMR in the environment – here defined as all settings which are not directly associated with a human or domesticated animal host, and not located in an indoor building where humans normally reside – is currently not well established (Pruden et al., 2021). The implementation of environmental AMR monitoring would greatly improve our understanding of the dissemination routes of resistant microorganisms outside of clinical and veterinary settings (United Nations Environment Programme, 2023). Importantly, the approaches to AMR monitoring in the environment would be directly translatable to other important settings for AMR transmission where surveillance could play a major role in curbing propagation of resistance.

2. Purposes of monitoring AMR in the environment

In clinical and veterinary AMR surveillance, the purpose is often to allow for interventions specific to certain pathogens and antimicrobial treatment regimes. Along the same lines, surveillance data can be used to take action in order to change use patterns and quantities of antimicrobials (VKM et al., 2022). The link to immediate intervention measures is harder to make for environmental AMR monitoring, and thus a key consideration in the implementation of such initiatives needs to be to decide which purposes such an effort should fulfill (Huijbers et al., 2019). Monitoring of sewage or sewage treatment plant effluents has often been motivated by a need to follow the AMR situation in the general population of, e.g., a city or urban area (Huijbers et al., 2020; Hutinel et al., 2019) in a relatively cost-effective manner. This practice has already proven to give valuable and actionable information about viral transmission among humans, most recently during the COVID-19 pandemic (Bonanno Ferraro et al., 2021), but also for decades in poliovirus surveillance. However, the goal of sewage surveillance is not directly related to risks associated with AMR within the environment; rather, the purpose is to use sewage as a bulk sample of the human

population in a confined area, such as a city. If one instead considers monitoring as a tool to assess risks to human and animal health associated with environmental development and transmission of resistance, it becomes fundamentally important to take into account the types of roles the environment plays regarding AMR.

In short, the environment can be important for two major AMR-related processes (Bengtsson-Palme et al., 2018). Firstly, the environment constitutes a means of dissemination for already resistant bacteria between humans, or between animals and humans. Resistant bacteria are released into the environment, for example, through sewage (Marathe et al., 2017), irrigation with reclaimed water (Christou et al., 2017) and via common agricultural practices, such as the use of manure (Jechalke et al., 2013; Sanz et al., 2022), as well as the application of treated sewage sludge as biosolids (Wolters et al., 2022). These bacteria can then be spread further with, e.g., animal migration (Ahlstrom et al., 2018; Jobbins and Alexander, 2015; Stedt et al., 2015). Evidence exists that these resistant bacteria subsequently can re-enter the human microbiome via the environment (summarized in Stanton et al., 2022), e.g. by ingestion of water contaminated with sewage during recreational swimming or water sports events (Leonard et al., 2018, 2015), the consumption of surface water irrigated fresh produce (O’Flaherty et al., 2019; Rahman et al., 2022), or more generally when sanitation is lacking.

In the second process, the environment acts as a source and facilitator for the evolution of AMR. Here, the environment can take at least four different roles: *i*) as a source of novel antibiotic resistance genes (ARGs) that can be mobilized into human pathogens (O’Toole, 2014), *ii*) in the selection for AMR, e.g., at sites polluted with pharmaceutical manufacturing waste (Bengtsson-Palme et al., 2014; Kristiansson et al., 2011), *iii*) in evolutionary processes leading to altered fitness costs associated with carriage of ARGs (Lin et al., 2018), and *iv*) as a secondary habitat for opportunistic pathogens that may acquire ARGs in the environment and subsequently spread them to human pathogens where they can be permanently incorporated during, e.g., antibiotics treatment (Bengtsson-Palme et al., 2018). The ARGs acquired in the environment may be of two types, which following Inda-Díaz et al. (2023) we designate as ‘established’ or ‘latent’. Some ARGs are already *established* and well characterized among human and animal pathogens, in which case additional recruitment from bacteria in the environment would only marginally contribute to their proliferation. Alternatively, ARGs can be *latent*, i.e. they have not previously been encountered in pathogens, and thus their acquisition from environmental bacteria has unknown but potentially much more severe consequences (Bengtsson-Palme and Larsson, 2015).

The motivation for monitoring AMR in the environment should be explicitly linked to one or more of these roles that the environment can play (Fig. 1). For the purpose of curbing the dissemination of already resistant microorganisms, it is crucial to determine the major sources that introduce resistant bacteria into the environment, which include sewage and sewage treatment plants, industrial sources, as well as agri- and aquaculture. In parallel, it would be important to characterize in what settings humans are exposed to significant numbers of resistant bacteria *from* the environment. These exposure processes are severely understudied and our understanding of the dissemination of resistant bacteria from environments to humans is currently mostly based on anecdotal reports (Stanton et al., 2022). Such exposure routes may involve the consumption of fresh produce (Rahman et al., 2022) or

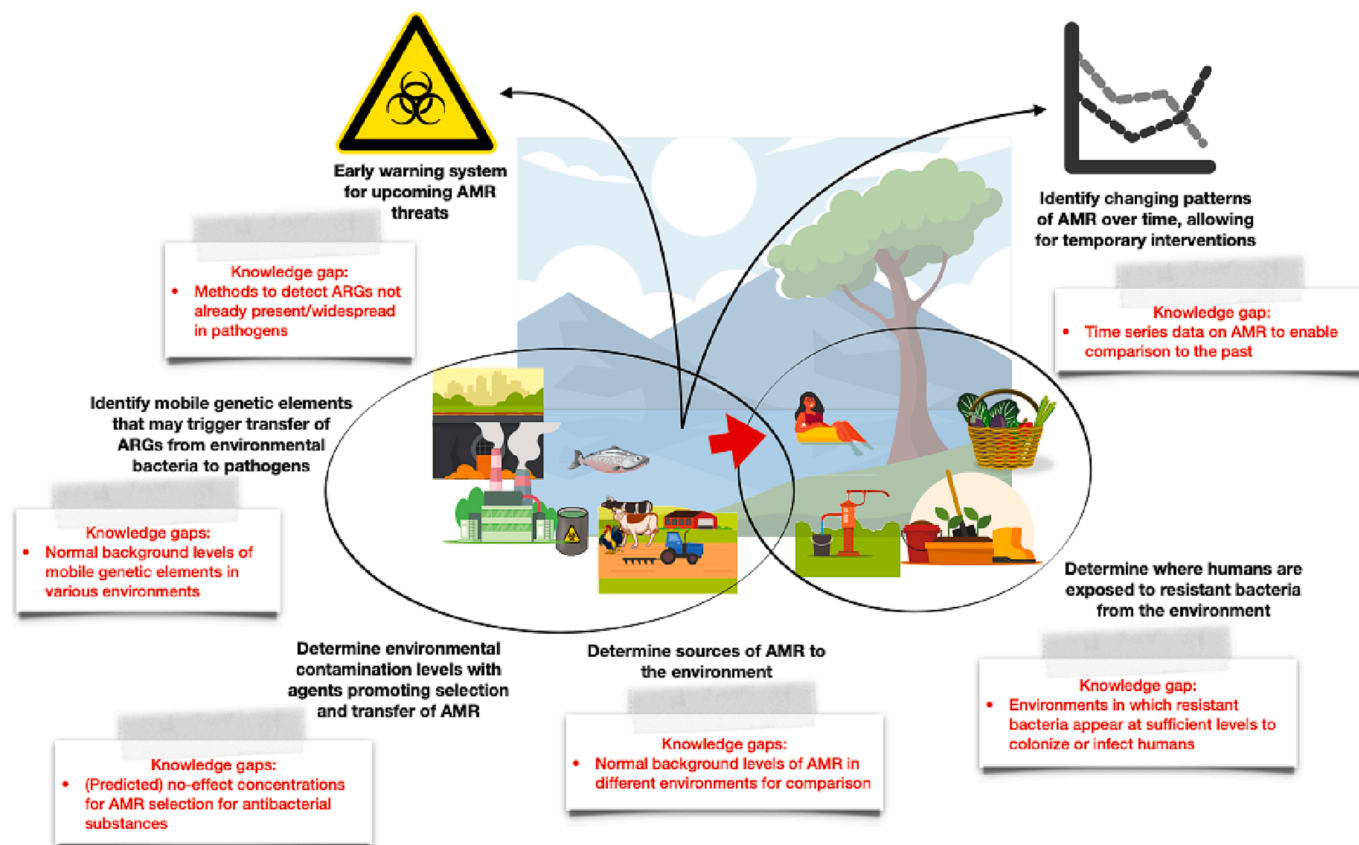


Fig. 1. Environmental monitoring for AMR can fulfill many different purposes. Monitoring could aim at determining the sources of AMR to the environment, including sewage, industrial pollution, agriculture and aquaculture. Another goal could be to identify where humans are exposed to resistant bacteria from the environment, for example by recreational swimming, drinking water, contact with animals or manure in agriculture and through the food chain. Environmental monitoring could also be used to detect changes in AMR over time, which can be used to assess if measures to reduce AMR in the environment have been effective, and would potentially also allow for temporary interventions. Furthermore, monitoring for AMR may aim to identify environments with the potential to select for resistant bacteria and ARGs. Finally, monitoring could be used as an early warning system for novel resistance mechanisms before they have appeared in or are widespread among human pathogens.

recreational swimming (Leonard et al., 2015). Whether these routes are actually the most important means of transmission from the environment to humans is presently unknown. Furthermore, AMR monitoring could be performed specifically to screen for changes in AMR abundance over time in particular environments, which could allow for temporary interventions, such as closing beaches or recommendations to boil drinking water. Surveillance for temporal changes would be relevant in the environments mentioned above, but may potentially also be highly informative in settings where such changes could indicate radical shifts in environmental AMR abundances, such as in the feces of wildlife (Ahlstrom et al., 2021; Arnold et al., 2016) or in pristine waters (Van Goethem et al., 2018). In other words, ARGs that change over time could function as indicator genes, and wildlife could be used as a sentinel setting for monitoring the spread of AMR into environments that are situated far from human activities (Plaza-Rodríguez et al., 2021). Finally, an ideal surveillance system should also incorporate some means of detecting upcoming AMR threats, including latent ARGs (Bengtsson-Palme and Larsson, 2015), particularly ARGs conferring resistance to last-resort antibiotics. Preferably, this system should also allow assessment of the risk that these ARGs could be transferred to pathogens. Such an early-warning system could allow acting on imminent AMR introductions before they become significant problems in clinical settings, e.g., by preventing (or at least delaying) their dissemination to humans or into the hospital environment.

Clearly defining the purposes of AMR monitoring allows for detailed answers to a number of follow-up questions regarding the choice of methods, environmental settings, and implementation scale (Box 1),

eventually ensuring feasible, useful and fit-for-purpose monitoring, while also being interoperable with other similar initiatives.

3. Monitoring AMR in the environment – Where to watch?

A major issue with monitoring of environmental AMR is that if the goal is not clearly defined, the mission can easily become monitoring of everything everywhere, which is obviously not a feasible task. For AMR monitoring to be worthwhile, it is critical to clearly specify relevant settings. Furthermore, sample sites and sample material should ideally correspond to control points where interventions could be implemented to curb the development or spread of AMR. One group of such control points is settings where resistant microorganisms and/or antimicrobials are released into the natural environment as a consequence of anthropogenic actions, particularly when these environments are closely connected to humans and animals (Larsson et al., 2018). Important examples of such settings include discharges from sewage treatment plants (Bengtsson-Palme et al., 2016; Lindberg et al., 2005; Michael et al., 2013; Pärnänen et al., 2019; Rizzo et al., 2013), raw sewage released directly into the environment (Bengtsson-Palme and Hess, 2019; Rahube et al., 2014), releases of waste material with high concentrations of selective agents, e.g., from antibiotic manufacturing (Gothwal and Shashidhar, 2016; Kristiansson et al., 2011; Larsson, 2014; Larsson et al., 2007; Milaković et al., 2019) or hospitals (Kraupner et al., 2021), as well as aquaculture (Cabello et al., 2016; FAO et al., 2006) and agriculture (Durso and Cook, 2014; Zhu et al., 2013). In all these settings there is a potential to limit the flow of resistant bacteria

Box 1

Questions that should be asked before implementing environmental monitoring of AMR.

- **Purpose of monitoring:** What is the motivation for doing this type of monitoring in this setting? What should be achieved? What type of risk should be assessed? What type of action would this enable?
- **Choice of methods:** What type of method(s) would be suitable to address these goals? Which of these methods (if any) are economically feasible? Which methods would deliver results within a useful timeframe for taking appropriate actions? What bacteria, antibiotics, ARGs and/or other genes should be targeted? What sampling strategy is appropriate (e.g., longitudinal studies, cross-sectional studies, point surveys)?
- **Targeted environments:** In what type of environment would monitoring for a given purpose be worthwhile? In which types of environments and settings is the proposed methodology feasible?
- **Intended users:** For whom would this monitoring strategy be applicable? Who would be able to use, implement and act upon this strategy given monetary, legal and practical constraints?
- **Integration potential:** How does the proposed monitoring strategy integrate with existing and future monitoring frameworks and efforts, particularly those already existing in the human and animal sectors? How can the resulting data be communicated and compared with other monitoring initiatives?

from human activities to the environment through regulatory, behavioral or technological interventions (Larsson et al., 2018; Pruden et al., 2013).

Another possibility to limit AMR spread to humans would be to restrict the flow of resistant bacteria from the environment to humans; in other words to impose restrictions to avoid human contact with resistant microorganisms from high-risk environments. Such measures would need to target situations in which humans interact with significant amounts of bacteria from environmental settings. Furthermore, from a cost-efficiency point of view, these control points should primarily include settings where samples are already taken for other surveillance purposes. Obvious examples of already ongoing surveillance are the compliance monitoring of drinking water and bathing water quality, under various organizational and legal structures (e.g. WHO/UNICEF MICS-6 module and the European Water Framework Directive). These surveillance points are closely tied to direct and actionable interventions, such as the recommendation to boil contaminated drinking

water and the closing of beaches with high levels of fecal contamination. In both cases, current surveillance is largely restricted to fecal bacteria, often using *Escherichia coli* or intestinal enterococci as indicators (Anjum et al., 2021). *E. coli*, in particular, is used across both human health and food safety monitoring programs. AMR in *E. coli* is also included in the WHO Global Antimicrobial Resistance Surveillance System (GLASS) main module and suggested in the WHO/FAO Codex Alimentarius guidelines on monitoring of foodborne AMR. Furthermore, *E. coli* carrying broad-spectrum beta-lactamases is the target pathogen across all biological compartments in the WHO GLASS One Health Module (Tricycle).

Conceptually, it would be possible to extend this monitoring to include specific markers of AMR, which has already been successfully demonstrated in small scale (Leonard et al., 2022, 2015). However, while conceptually straightforward, adapting such monitoring to include AMR might be complicated due to the scale at which it takes place. Furthermore, the regulatory monitoring of water quality does not

Box 2

Directions for future research to aid the implementation of environmental monitoring of AMR.

- **Establish how different AMR monitoring methods compare to each other:** This is a requirement for standardization for AMR monitoring in the environment, but it is not clear what such a standard should look like. Methodological choices should be based on what is feasible from, e.g., an economic perspective. Any eventual standard should incorporate targets that are informative across different settings and countries.
- **Extend pathogen-centric databases of ARGs with latent and proto-ARGs:** ARG databases are biased towards genes found in pathogens. To reduce this bias, they should be complemented by functionally characterized ARGs from, e.g., functional metagenomics. ARG databases also need to be continuously maintained so that newly discovered ARGs can be added. More effort should also be devoted to exploring environmental microbiomes for novel ARGs and populating databases with these.
- **Determine the locations and type of environments relevant for AMR monitoring:** To reduce costs, utilizing already existing environmental monitoring should be prioritized, as should locations integrated into operating or planned surveillance programs. More efforts should also be made to identify additional pathways for AMR transmission through the environment.
- **Study the environment as a source and transmission route for AMR:** Stratify risks associated with ARGs found in the environment. Define typical levels of AMR in different environments. Identify spatiotemporal patterns of AMR in the environment. Determine under what circumstances it would be beneficial for microbes to carry ARGs in the absence of human impact.
- **Identify settings where the relationship between fecal indicators and AMR is absent:** These environments are important as they deviate from the expected baseline of AMR. It should also be determined to what extent there is a relationship between fecal pollution and latent ARGs. This knowledge can aid in identifying situations in which it would be helpful to investigate a microbial community for resistance to specific antibiotics. This would allow for simple indicators to be used to show when to study a particular environment in more detail.
- **Identify origins for more ARGs:** This knowledge will be instrumental in preventing the emergence of new forms of AMR in pathogens in the future. Tracing the evolutionary history of established ARGs can help understanding where the future threats from latent ARGs are most relevant.

currently incorporate molecular techniques. On the other hand, if already established surveillance could be expanded and data from monitoring systems that are now maintained for different separate purposes could be aggregated, we would already have a fairly broad environmental surveillance network in place, albeit restricted to a limited set of selected, but likely highly relevant, environments (See Box 2).

If the major goal of environmental AMR monitoring is to track the effects of human activities on the level of AMR in natural settings, several sampling strategies are available. One could be sampling of sites where wild animals repeatedly defecate and urinate, so called wildlife animal latrines, in order to track changes over time and potentially link them to human activities (Pesapane et al., 2013). This type of monitoring could provide information about AMR transmission in wildlife species in a non-invasive way, although sampling AMR for this purpose is currently rarely explored. Another sampling and analyses strategy could be to include AMR into already established ambient water quality monitoring, such as the UN Environment Global Environment Monitoring system for freshwater (<https://www.unep.org/explore-topics/water/what-we-do/monitoring-water-quality>), and the monitoring of different aspects of soil and which has already included measuring the level of certain antibiotic residues in the European Land Use/Cover Area statistical Survey Soil (Orgiazzi et al., 2018).

Finally, if the goal is to assess the risks that antimicrobial agents and AMR pose on the environment itself, different targets and methodologies must be applied, depending on what outcome is of main interest. Examples of outcomes to study are changes to microbial ecology, or toxic and hormonal disruption effects on higher order organisms. When studying microbial ecology, culture-independent methods are necessary, because non-cultivable microorganisms represent the great majority of the planet's biodiversity. High-throughput molecular technologies are essential to understand genetic diversity, population structure and metabolic interactions (Michán et al., 2021). These methodologies have been applied on different ecosystems such as soil and water sediments, to assess the effect from human and animal sectors on, e.g., fresh water bacterial communities (Kraemer et al., 2022). However, discussion on the risks on the environment itself is a complicated and wide-ranging topic, which deserves its own in-depth analysis.

4. Needs prior to implementation of environmental AMR monitoring

To enable the monitoring strategies mentioned above, there are several urgent data needs (Fig. 1). In order to understand which settings are important sources of AMR to the environment and to assess if background levels are exceeded, we need knowledge of the baseline ('normal') levels of AMR in different environments. Similarly, background level data is also crucial for understanding what settings would result in a significant exposure of AMR to humans and animals. Without information on the typical abundance ranges for both resistant microorganisms and ARGs, any single measurement of AMR in a given environment would be lacking context, hence making it impossible to associate it with a risk to human or animal health. For particular sampling points, it would be possible to contextualize measurements through time series, which would also enable the type of temporary interventions discussed earlier. However, for broad screening across many different samples from different locations, time-resolved sampling might simply not be a feasible option. In these cases, detection of deviations from known typical background levels may be used to identify which sampling points may warrant further investigation, for example to discern the point sources of particular types of resistance. Unfortunately, such data is mostly lacking, although recent efforts to collect data through meta-analysis have yielded some useful information (Abramova et al., 2023; Keenum et al., 2022). It is particularly interesting to note that for the vast majority of established ARGs already circulating among human pathogens, the typical environmental relative abundance level

ranges from 10^{-5} to 10^{-3} copies per bacterial 16S rRNA sequence (Abramova et al., 2023). For example, relative abundance of tetracycline genes *tetA*, *tetB* and *tetG* from human impacted environments, such as aquaculture fishponds, urban rivers and pig farms, average to 10^{-3} copies per bacterial 16S rRNA. In contrast, relative abundances of *tetA* and *tetG* genes in comparatively unimpacted environments in High Arctic (McCann et al., 2019) remain below 10^{-6} copies per bacterial 16S rRNA, while *tetB* was not detected at all, suggesting that these genes can serve as good indicators for anthropogenic pollution (Abramova et al., 2023). Similar patterns were observed for the beta-lactamase resistance gene *blaTEM* and the sulfonamide resistance gene *sulI*, which were detected at 10^{-7} copies per 16S rRNA in soil samples from tundra, glaciers, and polar deserts (Hayward et al., 2018; McCann et al., 2019), while reaching up to around one ARG in a thousand bacterial cells in soil samples from agricultural fields and residential areas (Knapp et al., 2017). Importantly, it is virtually impossible to find a "pristine" environment devoid of human activity. Even remote locations such as high mountains or Antarctica are still affected indirectly by human activity through atmospheric depositions or birds and animals which act as vectors of ARGs and resistant bacteria (Hayward et al., 2018; Hwengwere et al., 2022; Segawa et al., 2012). Despite that the *sulI* gene showed very low abundance in High Arctic samples, in sediments from a seemingly pristine origin in the Rocky Mountains, without major human activities, it was detected at an abundance of 10^{-2} copies per 16S rRNA (Pruden et al., 2012). Similar levels were also found in soils from a nature reserve in Lian Mountain, China (Wang et al., 2014) and alpine lakes without any wastewater plants or hospitals in their vicinity (Czekalski et al., 2012).

Furthermore, it is not known whether the abundance range of 10^{-5} to 10^{-3} ARG copies per bacterial 16S rRNA would be typical also for latent ARGs, or if these are, e.g., rarer or show larger variation. However, as more latent ARGs are identified, previously sequenced and deposited metagenomes can be used for a form of 'retrospective monitoring' to identify how common they are in different environments, and what bacteria host them (Inda-Díaz et al., 2023). It would also be possible to trace when they originally appeared in high-risk genetic contexts, such as in plasmids, transposons or prophages, as well as if or when they first appeared in pathogens or high-risk environments. All this information could inform risk management and guide decisions on what actions to take with regards to latent ARGs in the environment. However, an important caveat to this is the fact that metagenomic sequencing efforts need to be deep enough; sequencing upwards of a hundred million reads per sample would be necessary to detect relevant genes in archived datasets, as they can be assumed to be fairly rare, which in turn also means that the costs associated with generating new data sets aiming to detect latent ARGs can – at present – be prohibitive for routine monitoring.

Therefore, when monitoring is used as an early warning tool to detect and quantify these novel forms of ARGs, we would need new methods that can identify latent ARGs with high accuracy, determine and rank their hazard potential in terms of the consequences to human health if they spread to pathogens, and prioritize which ones should be included in monitoring. A number of methods exist for detecting novel ARGs based on sequence or structural similarity, which can be followed up by phenotypic verification through gene synthesis (Berglund et al., 2019; Ruppé et al., 2019). However, these methods are still limited to known structural classes of ARGs. To discover ARGs in gene families not yet known to be involved in AMR, further method development would be needed. Potentially, large-scale experimental approaches, such as functional metagenomics (Allen et al., 2009; Lang et al., 2010; Marathe et al., 2018), may be the only way forward to discover entirely new classes of ARGs. These methods are, however, labor intensive and likely out of the question for routine AMR monitoring in the environment. Despite this, they offer informative and complementary information to culturing, qPCR and DNA sequencing. Moreover, because they are labor intensive, any new ARGs characterized by these methods should be

added to existing ARG databases to allow future monitoring for them using less laborious methods.

Aside from these considerations, monitoring for AMR in the environment would be significantly more feasible if it could be based on surveillance systems that are already in place, and that already apply some form of microbiological analyses. Previously mentioned examples of such surveillance systems are those to monitor drinking water quality, acceptable bathing water quality at beaches, or surveillance that takes place in food production as well as imported food products. In all these circumstances, also targeting AMR would have modest marginal costs, given methods are cheap, fast and easy-to-use. If not, including AMR and antimicrobial residues into monitoring systems that hitherto have mainly focused on physical and chemical characterization of environmental samples would be more elaborate and costly. Also, it is worth noting that most of these systems are in place to detect and prevent infections or the effects from toxic substances. In the case of AMR, the goal is partially to prevent infections caused by bacteria carrying AMR, but also to prevent the transfer of ARGs from non-pathogenic bacteria to human pathogens. Although quantification of coliforms is already included in freshwater monitoring in the UNEP GEMS/Water system, many countries do not perform such analyses yet and including the cultivation *E. coli* with subsequent AMR-testing would further add to this workload.

5. Harmonizing protocols for comparing AMR data

Both for resistance that is already established in human pathogens and for latent ARGs, it remains critical to know how well measurements obtained using one method correspond to other ways of quantifying resistance, such as how selective culturing compare to the abundance of ARGs determined by qPCR or shotgun metagenomics. At present, little evidence exists on what types of bacteria and resistance mechanisms that best predict the overall abundance and diversity of resistant bacteria and ARGs in a given environment. Similarly, data regarding what specific ARGs are most predictive of the total ARG content in a microbial community is limited (Bengtsson-Palme, 2018), and the correspondence between shotgun metagenomic ARG abundances and qPCR-based measurements from the same samples has only been explored in limited settings (Crossette et al., 2021; Heß et al., 2019).

Considering the variety of possible methods for monitoring AMR in the environment, there is a need for harmonizing the different efforts currently being tried and implemented worldwide to make them comparable and interoperable. Such a harmonization effort would necessitate comparison of several different methods on the same set of samples, preferably sampled from a variety of different environments and geographical areas. Which types of bacteria should be selected for culturing in such a scenario and for which antibiotics resistance should be tested is an open question. In order to make sure there is a minimum degree of compatibility between existing protocols, a good starting point would be the WHO GLASS and the WHO One Health Module (Tricycle protocol) (World Health Organization, 2021a, 2021b). Yet, to ensure environmental relevance, they should be extended to additional bacterial species and resistance patterns beyond the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*). Similarly, it is far from obvious which genes should be targeted using qPCR. Previous studies provide some useful clues as to which genes may be the most informative, and from these the selection could subsequently be narrowed down to the most meaningful targets (Abramova et al., 2023; Bengtsson-Palme, 2018; Berendonk et al., 2015; Keenum et al., 2022). Alternatively, one could focus on important ARGs among the WHO priority pathogens (World Health Organization, 2017), but again the precise selection of genes is strongly related to the purpose of monitoring.

In the case of shotgun metagenomic sequencing, the target gene selection is linked to the choice of database, which can be amended or

changed over time (Angers-Loustau et al., 2018). Furthermore, it would also be important to select appropriate bioinformatic methods for this analysis, and make sure that the same methods are consistently applied on every dataset in the comparison (Bengtsson-Palme et al., 2017). Metagenomic analysis could be used to pinpoint which predictors give the most relevant information about datapoints obtained using other methods, i.e. what measures are informative of each other, and which ones provide the best overall overview of the resistance situation in a given environment. Conceptually, this could be condensed into a kind of composite AMR score, although it is unclear if such a score would be relevant and meaningful enough, or even feasible.

Another crucial aspect in terms of harmonization of AMR monitoring efforts is that the contextual data describing the samples is consistently collected, recorded, and subsequently made accessible. Similarly, it would be desirable to make sure that the same methods are applied uniformly, so that data from disparate studies and monitoring programs can be jointly compared, synthesized, and analyzed (Davis et al., 2023; Kormos et al., 2022; Milligan et al., 2023). However, while such standardization should be the ultimate goal for environmental AMR monitoring, it is not clear at present what those standards should be, making it premature to impose a standard at this moment in time.

6. Environmental baseline for AMR

As mentioned earlier, another prerequisite to contextualize environmental monitoring of AMR is having knowledge of the typical background levels of resistance in different environments. There have been some efforts to address this at a global scale, usually using reanalysis of public metagenomic datasets (Bengtsson-Palme, 2018; Cuadrat et al., 2020; Nesme et al., 2014; Pal et al., 2016), but also using meta-analysis of qPCR studies (Abramova et al., 2023; Keenum et al., 2022) (Fig. 2). Given that different monitoring methods can be harmonized and made somewhat comparable (see above), it would be possible to integrate metagenome data from public resources (Coelho et al., 2022), the above-mentioned collections of qPCR studies from the literature, and the extensive monitoring data based on culturing from clinics (Giske et al., 2013; Poulou et al., 2014; World Health Organization, 2021a), livestock (domestic) animals (European Food Safety Authority et al., 2019; FAO, 2019; Schrijver et al., 2018) and environmental settings (Anjum et al., 2021; Flach et al., 2021; Grevskott et al., 2021). Another important data source for comparison is global sewage monitoring (Aarestrup and Woolhouse, 2020; Hendriksen et al., 2019), although arguably this type of monitoring reflects the local AMR situation in humans rather than that of the environment. While the reports from clinical and animal monitoring can only scratch the surface in terms of AMR in these settings, they may still be able to provide an overview for the environmental AMR baseline. However, since the actual baseline is virtually unknown, it is very hard to know to what extent this link between clinics, animals and the environment holds true. It is notable, that much of this resistance monitoring data is binary rather than quantitative, as in either resistant bacteria are present (detected) in a sample or go undetected. It is unclear how this type of data can best be integrated as a predictor of overall AMR in an environment. However, it is likely that given a suitable modeling approach, such binary datapoints could be used as indicators of settings or specific sites that warrant further investigation using more specific methods.

7. Selection of high-priority monitoring targets

As discussed earlier, a major challenge in the process of implementing environmental monitoring for AMR is choosing methods and targets that would provide the most information. However, the amount of information gained from a given data point is not the only important consideration in this selection process. In many cases, it would make sense to perform surveillance for ARGs of particular clinical interest, such as those known to cause difficult-to-treat infections (Berendonk

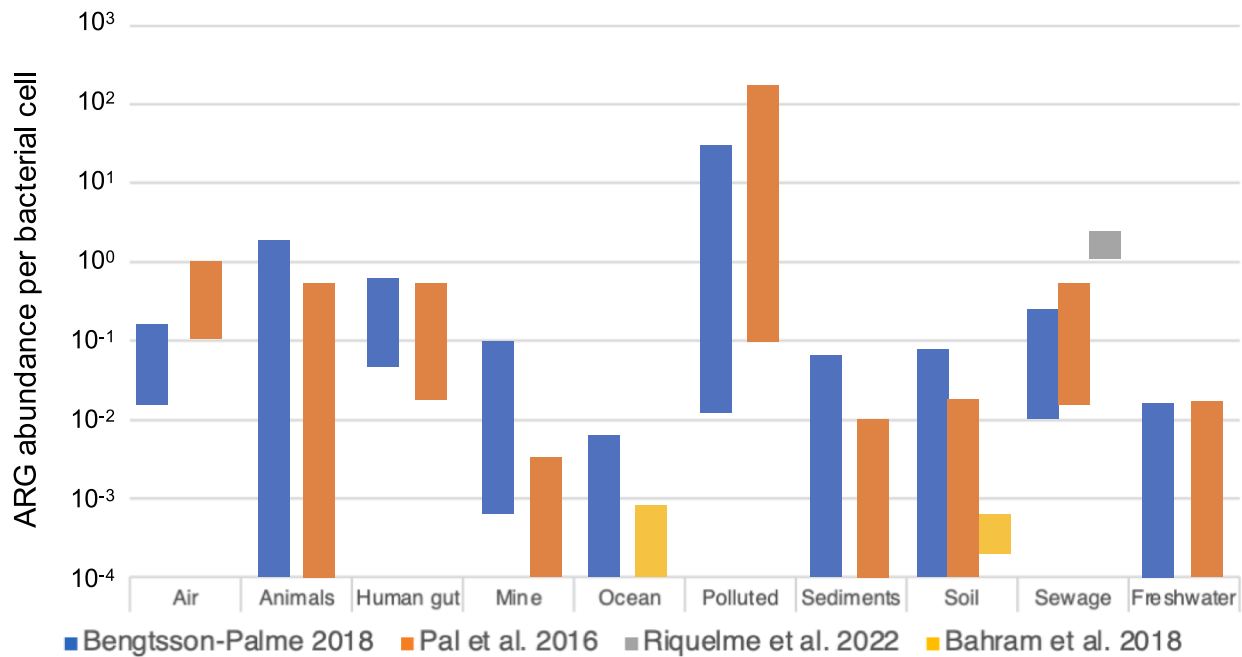


Fig. 2. Typical ranges of total ARG levels in different types of environments, collected from large-scale efforts analyzing a wide range of metagenomes (Bahram et al., 2018; Bengtsson-Palme, 2018; Pal et al., 2016; Prieto Riquelme et al., 2022). Abundances are given as ARG copies per bacterial cell, usually quantified comparing to 16S rRNA. As different studies used different, but similar, abundance metrics the ranges should be seen as indications, not as absolute measurements. ARGs are most common among bacteria in highly polluted environments, mostly driven by sediments subjected to waste from pharmaceutical production (including high levels of antibiotics). Furthermore, ARGs appear in similar relative abundances in air, animals, sewage and the human gut. Levels in surface water, sediments and soil are overall considerably lower. Note that even though the relative abundance of ARGs is high in e.g. air, the total number of cells is likely to be orders of magnitude lower than in, e.g., the human gut.

et al., 2015). To determine which ARGs are of high priority from a clinical point of view, it can be useful to consider whether they are associated with mobile genetic elements, if they encode resistance towards last resort antibiotics such as carbapenems, or whether they are present among the ESKAPE pathogens (Pendleton et al., 2013). Importantly, though, these ARGs of particular clinical relevance are likely to be rarer to be encountered, which may result in that they are often not detected, and therefore would be less suited to inform modeling studies of environmental AMR.

There can also be ecological reasons to prioritize certain ARGs, such as whether they are enriched in human-associated environments, present in environments closely connected to humans, often found across several different types of environments, and whether they could be indicators able to differentiate between human, animal and environmental origin of AMR. One example of the latter is colistin resistance, which should primarily be enriched in agricultural environments due to the very limited use of colistin in humans and its relatively high use in animals (Kempf et al., 2016). Finally, there could also be practical or historical reasons to prioritize certain ARGs. For example, there is already abundant data and standardized methods for detecting ESBL-genes, particularly in *E. coli*. These methods are connected to the WHO GLASS objectives (World Health Organization, 2021a), which also points to their potential for interoperability. There are also ongoing surveillance efforts, particularly in domestic animals, which target specific bacteria and types of resistance (European Food Safety Authority et al., 2019; FAO, 2019; Simjee et al., 2018), and there might be reasons to include at least some of these in environmental monitoring. In the ideal situation, these concerns should be weighted together with the amount of information that can be gained from a given endpoint to guide the selection of monitoring targets.

8. Risk environments for ARG emergence

One major goal of environmental AMR monitoring could be to identify environments not only associated with dissemination of resistance, but also with a particular risk for emergence and selection for novel forms of AMR. To do so, data on the abundances of latent ARGs, not typically occurring in human pathogens, are needed. Since there are currently no effective methods for large-scale identification of latent ARGs which are not homologous to established ARGs, these genes would be inherently hard to detect. That said, in an effort to pinpoint environments of particular risk, soil, water and environments contaminated with waste from antibiotic production was shown to have high abundances of latent ARGs (Bengtsson-Palme, 2018). However, it should be noted that the selection of latent ARGs in that study was limited to the few ARGs that have been identified outside of pathogens using experimental methods (Wallace et al., 2017). In addition, wastewater has been pointed to as an environment with both high abundances of latent ARGs (Inda-Díaz et al., 2023) and bacterial species thought to be the origin of established ARGs (Berghlund et al., 2023). It would be possible to integrate high-quality bioinformatics predictions of novel ARGs into monitoring, as those have been shown to accurately predict functional ARGs in more than 70% of cases (Berghlund et al., 2017; Boulund et al., 2017; Ruppé et al., 2019) (Fig. 3). As the likelihood of transfer of ARGs between bacteria is a clear risk factor for resistance development in pathogens, the abundance of mobile genetic elements in an environment, as well as which ARGs that are coupled to what transferrable elements, should also be taken into account when defining risk environments (Bengtsson-Palme et al., 2014; Ghaly and Gillings, 2022; González-Plaza et al., 2019). Here, novel methods that quantify the linkage of an ARG to mobile genetic elements could deliver valuable insight for risk assessment (de la Cruz Barron et al., 2022). One possibility could be the use of Hi-C sequencing, in which the hosts of mobile

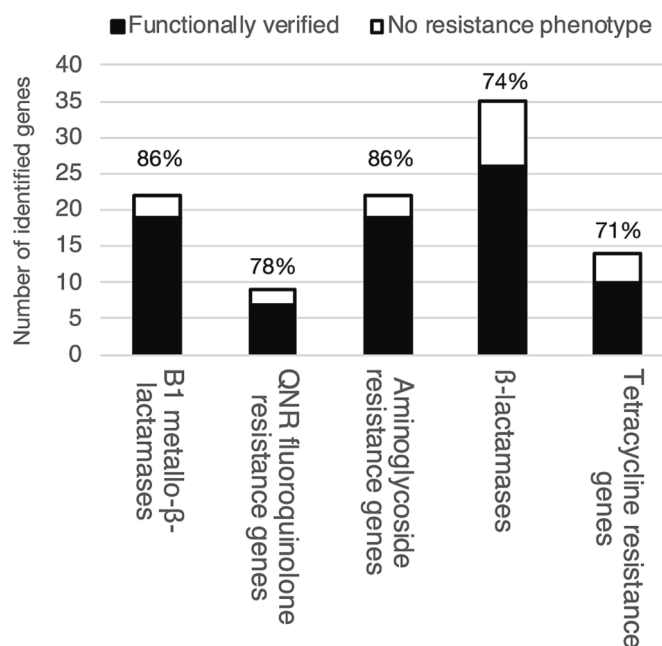


Fig. 3. Proportions of functionally verified predicted resistance genes using different high-quality bioinformatics prediction methodologies. The number of tested predictions that did not show a resistance phenotype are shown in white. The first bar shows results of the methodology of [Berglund et al. \(2017\)](#) applied to class B1 metallo-beta-lactamases, the second the (very similar) methodology of [Boulund et al. \(2017\)](#) applied to *qnr* genes, and the rightmost three bars represent the performance of the [Ruppé et al. \(2019\)](#) method for three different ARG classes. Data was aggregated from what was reported in the original sources.

ARGs can be identified through chromatin conformation capture, when DNA in individual cells is crosslinked by formaldehyde ([Kalmar et al., 2022](#); [Kent et al., 2020](#)). However, while this method would be able to identify the plasmids carrying mobile ARGs in individual hosts, it is still a somewhat expensive and laborious protocol for routine AMR monitoring.

Another aspect pointing to high risks for AMR emergence and selection is selective levels of antibiotics or other chemicals that could induce enrichment of resistant bacteria and ARGs in the environment or promote their transfer between bacteria on mobile genetic elements ([Larsson and Flach, 2021](#)). This points to a need for *i*) concentration data for antibiotics and other selective agents, such as antibacterial biocides and heavy metals, in a variety of environments, and *ii*) establishment of the concentrations of these substances that lead to selection for AMR or increased transfer of genetic material between bacteria. Although concentration data for antibiotics have been collected in many parts of the world ([Chow et al., 2021](#)), more data would be necessary to obtain a comprehensive picture, particularly for other selective compounds than antibiotics. In terms of concentrations that would drive AMR selection, there is very little experimental data to directly measure this in complex microbial communities in environmental settings. For single species, selection for resistance has been shown to take place at much lower concentrations than those that inhibit growth ([Gullberg et al., 2014, 2011](#)). This is also the case in laboratory experiments on complex microbial communities ([Kraupner et al., 2018](#); [Lundström et al., 2016](#)). A useful starting point would be to integrate concentrations predicted not to promote resistance development in complex microbial communities ([Bengtsson-Palme and Larsson, 2016](#)), although they are based on growth inhibition and whether they are protective enough for other environmental effects have been debated ([Le Page et al., 2017](#)). The minimal selective concentrations concept for single species has been used to consolidate these predictions ([Andersson and Hughes, 2012](#)).

However, due to a lack of data on MICs for many antibiotics, but even more so a lack of minimal selective concentrations for all except the most common pathogenic species-antibiotic pairs, the environmental relevance of these efforts is uncertain ([EFSA Panel on Biological Hazards \(BIOHAZ\) et al., 2021](#)). Notably, almost all available data on inhibitory and selective concentrations concern antibiotics only, while for biocides, virtually no such data exist ([Wieck et al., 2016](#)). Similarly, almost all our mechanistic knowledge of AMR selection is based on laboratory evidence. Our current understanding of exactly how resistance is selected for or against in complex microbial communities in the environment, and which factors matter for these selection processes, is extremely scarce ([Bengtsson-Palme et al., 2018](#)). For example, when a single strain population was embedded in a complex microbial community, it needed an increased antibiotic concentration to select for resistance as compared to single strain experiments ([Klümper et al., 2019](#)). Finally, antibiotics and biocides have both been shown to induce transfer of genetic material between bacteria ([Hastings et al., 2004](#); [Jutkina et al., 2017](#); [Seier-Petersen et al., 2014](#)). However, it is worth noting that the concentrations inducing horizontal gene transfer of ARGs may differ from the ones directly selecting for resistance.

An important aspect in the assessment of risks associated with both established and latent ARGs is the genetic context in which they are located ([Martinez et al., 2015](#)). Particularly, ARGs located on mobile genetic elements, such as plasmids or integrative chromosomal elements, are easily transferred between bacteria, including between environmental bacteria and human pathogens. In addition, genes located next to transposases or integrases may easily be moved between plasmids or between the chromosome and plasmids ([Gillings, 2014](#)). It is therefore important to include, in addition to ARG abundances, such aspects in the risk assessment of AMR in the environment ([Bengtsson-Palme et al., 2018](#); [Martinez et al., 2015](#); [Zhang et al., 2021](#)).

In order to establish risks to human and animal health associated with the environmental emergence or presence of latent ARGs, it is also important to consider the connectivity between a given environment and the human or animal population ([Bengtsson-Palme et al., 2018](#)). Such connectivity has many dimensions, including geographical closeness in space ([Bahram et al., 2018](#)), but also in time. Furthermore, geographical features, such as topology, also impact the dissemination patterns for AMR ([Hooban et al., 2021](#); [Schar et al., 2021](#)), as do habitat fragmentation and connectivity created by humans, such as trade routes and national borders ([Bengtsson-Palme et al., 2015](#); [D'Souza et al., 2021](#); [von Wintersdorff et al., 2014](#)). Finally, it is conceivable that one could use mathematical modeling approaches including these factors to identify environments that would be associated with high risks for the emergence of novel ARGs. However, recent efforts to do so have been thwarted by a lack of precision in the data that would be needed to determine the parameters of the model ([Bengtsson-Palme et al., 2021](#)). Consequently, such approaches are not likely to be useful in risk management for the next couple of years.

9. Watchlist for future ARGs and other targets

For emerging ARGs, it would be useful to create a ranked watchlist for upcoming potential AMR threats. Such a watchlist would encompass latent ARGs which are of concern for one or several reasons, which could include *i*) high-level resistance observed in experiments, particularly to critical antibiotics ([World Health Organization, 2019](#)), *ii*) indications of broad spectrum of activity or poor clinical outcomes when the gene is detected in pathogens, *iii*) indications that the gene is located on a highly transferrable mobile genetic element, *iv*) low fitness cost for the bacteria carrying the gene, and *v*) short phylogenetic distance between the original host and pathogens.

Outside of the high-priority latent ARGs ranked highest on this watchlist, there are additional genes of interest, including so-called 'proto'-ARGs ([Kim and Cha, 2021](#); [McArthur and Wright, 2015](#)). Proto-ARGs may be of lower priority as they do not show full-blown

resistance phenotypes, but could include genes consistently found in certain environments and which are closely related to established ARGs. It could also encompass ARGs against antibiotics in the development pipeline that have not yet made it to the market. The latter would probably be hard to identify, but if they could be identified, for example by binding site modelling or functional metagenomics, surveillance for proto-ARGs should be a high priority to enable actions to preserve the efficacy of new antibiotics substances for as long as possible. The list of targets of interest could also contain certain mobile genetic elements, particularly in specific high-risk environments such as biofilms or freshwater under poor sanitary conditions. Furthermore, specific high-risk clones for mobile AMR could be added, based on the knowledge that there is a close relationship between certain bacterial lineages and the frequency of ARGs (Brinda et al., 2020; Cooper et al., 2020).

Having access to such a watchlist of upcoming AMR threats of concern would allow for adjusting sewage monitoring to include genes from this list. This way, it can be detected when and where such ARGs disseminate into the human population. Such sewage surveillance for AMR has seen a rapidly increasing interest (Hendriksen et al., 2019; Huijbers et al., 2020; Hutinel et al., 2019), and sewage epidemiology is on the verge of being introduced broadly in many places globally after the COVID-19 pandemic (Aarestrup et al., 2021; Aarestrup and Woolhouse, 2020; Pruden et al., 2021). Incorporating an environment-based watchlist for ARGs into these efforts would provide an early warning about emerging AMR before these genes are widespread in clinical settings (Flach et al., 2021). Finally, this watchlist would also aid in designing diagnostic tests that can be used to detect emerging ARGs in the clinical setting.

10. Discussion

The most severe consequences of AMR development in bacteria can be seen in the clinic, with dwindling numbers of functional antibiotics complicating both treatment of infectious diseases as well as routine procedures such as surgery, cancer treatment and the delivery of newborns where antibiotics are used prophylactically. Although this is where we can observe the direct impact of AMR, there is strong evidence that many ARGs originated in nature, possibly in non-pathogenic, non-human associated bacteria, and were subsequently transferred to pathogens after the introduction of antibiotics to treat infections (Bahram et al., 2018; D'Costa et al., 2011; Ebmeyer et al., 2021; Forsberg et al., 2012; O'Toole, 2014). At the same time, the particular origin is known only for a minority of ARGs, and it should be a priority to identify the origins for more of them as a part of preventing the emergence of AMR in pathogens.

It is also important to put the findings of environmental AMR surveys into context. Are all identified ARGs in the environment indicative of the same risks (Bengtsson-Palme and Larsson, 2015; Martinez et al., 2015)? What are the normal ('natural') levels of AMR in different types of environments? When may AMR in the environment pose a risk to human health? It is also worth considering the relevance of AMR in natural settings. Are there spatiotemporal patterns of environmental AMR that indicate current trends that may eventually reach clinical significance through spillover? Under what non-anthropogenic circumstances would it be beneficial for microbes to carry ARGs? It is likely that at least some ARGs have evolved and been maintained to tolerate antibiotic substances produced by other microorganisms, or potentially by the producers themselves to protect against their own antibiotics (Aminov, 2009; Bahram et al., 2018; D'Costa et al., 2011). It is thus important to characterize in what situations AMR is a problem and under what circumstances it might not be of great concern in terms of human health risks.

Clearly, there are several hurdles to overcome before monitoring for AMR in the environment can be broadly implemented, at least outside of sewage surveillance (Aarestrup and Woolhouse, 2020; Flach et al., 2021). Several methods have been used to investigate AMR in sewage,

most prominently selective culturing (Grevskott et al., 2021; Huijbers et al., 2020; Hutinel et al., 2019), shotgun metagenomics (Bengtsson-Palme et al., 2016; Fresia et al., 2019; Hendriksen et al., 2019; Rodríguez et al., 2021), and qPCR (An et al., 2018; Karkman et al., 2016; Pärnänen et al., 2019; Wang et al., 2020). In the interest of cost-efficient monitoring of AMR in the environment, we need to consider whether all these methods are necessary to gain a complete picture of AMR in the environment. However, since they all come with pros and cons, it is difficult to say that one is always preferable over the others. Culturing is very sensitive in terms of detecting resistant bacteria at a low abundance, and has the benefit of directly measuring phenotypic resistance. Unlike qPCR and metagenomics, selective cultivation can also directly link a specific type of resistance to a specific host with certainty. Emulsion paired isolation and concatenation PCR (epicPCR), which involves the encapsulation of individual cells and fusion of ARGs to phylogenetic markers during the PCR ahead of subsequent sequencing, is another method for linking target ARGs with their hosts (Spencer et al., 2016). However, while epicPCR has been proven to be valuable in determining the host range of ARGs in complex environmental samples and answering specific ecological questions regarding ARG distributions (Hultman et al., 2018), its highly technical and time-demanding sample preparation makes it an unlikely candidate for implementation in high-throughput monitoring campaigns. Further, the host identification done by epicPCR is currently restricted to the genus level, as only a short fragment of the highly conserved 16S rRNA gene can be used for host identification (Hultman et al., 2018). Another promising technique to link mobile ARGs to specific hosts is Hi-C sequencing (Kalmar et al., 2022; Kent et al., 2020). However, similarly to epicPCR this technique also requires substantial expertise and is comparably expensive. Therefore, culture-based methods remain a reliable and important way to directly connect ARGs to their hosts at the species level. Yet, selective culturing is limited to the specific bacterial species and antibiotics that are being tested for.

Similarly, qPCR-based methods are restricted to a pre-established set of gene targets, but have the benefit of being extremely sensitive in detecting these targets even at very low concentrations. Furthermore, as qPCR does not require the cultivation of bacteria, it can also detect ARGs among non-cultivable or slow-growing bacteria, which may be of particular interest in the environment. Shotgun metagenomics share this last benefit with qPCR, but has the drawback of not being very sensitive to rare bacteria or genes, often just scratching the surface of the studied microbial community even with very high sequencing efforts (Bengtsson-Palme et al., 2015). However, in contrast to culturing and qPCR, shotgun metagenomics is open-ended, in the sense that it does not target any pre-established set of genes, antibiotics or bacteria. That said, ARGs still need to be in a database to be detected in any convenient manner. In general, ARG databases are biased towards genes found in pathogens and in culturable bacteria, which do not represent the majority of bacterial diversity. To reduce this bias, it is important to combine pathogen-centric databases with databases containing functionally characterized ARGs from, e.g., functional metagenomics, such as ResFinderFG (Gschwind et al., 2023). Generally speaking, no single ARG database currently in existence is comprehensive enough to cover the diverse environmental resistome, and databases would need to be continuously maintained to also include newly discovered ARGs. A great benefit of metagenomics is that the data can be saved and then re-analyzed by bioinformatic tools at a later stage for newly discovered forms of resistance. This allows for a kind of 'retrospective monitoring' for AMR that is not possible using culturing or qPCR.

Taken together, the methods suitable for environmental AMR monitoring seem to be complementary rather than redundant, which is not optimal from a cost-savings perspective. Still, certain redundancies between the targeted endpoints exist. For example, the general abundance of cultured *E. coli* in environmental samples is strongly correlated to the levels of resistant *E. coli* (i.e., in the majority of cases the proportion of resistant *E. coli* in a mixed population is fairly stable) (Ott

et al., 2021), although the ratios will be somewhat dependent on the local resistance situation in the human population (Huijbers et al., 2020). Thus, general *E. coli* abundance, which is already monitored at, for example, many public beaches (Havs- och vattenmyndigheten, 2022; Tiwari et al., 2021) could be a reasonable starting point to determine AMR risks, even without performing any resistance testing. In a similar fashion, the overall ARG abundance in environmental samples is often directly correlated to fecal contamination (Karkman et al., 2019). Thus, indicators of fecal pollution, including *E. coli* or the crAssphage (Edwards et al., 2019; Guerin et al., 2018), could be useful proxies for AMR pollution. That said, the correlation between AMR and fecal pollution is not always valid. For example, in lake sediments polluted by pharmaceutical production waste, fecal indicators could not explain the high levels of ARGs (Bengtsson-Palme et al., 2014). Similarly, in mining environments, high ARG abundance coupled to low ARG diversity could not be explained by fecal indicators (Yi et al., 2022). Further, in pristine environments with relatively low ARG abundance, such as groundwater, ARG dynamics are rarely correlated with fecal indicators (Kampouris et al., 2022). These studies highlight that it is crucial to identify the settings where the relationship between *E. coli* or other fecal indicators and AMR is absent. It is also unknown as to what extent there is a relationship between fecal pollution and latent ARGs, although such a positive correlation seems to exist to some degree, in at least some environments (Bengtsson-Palme, 2018). Another important factor is to determine in what situations or under what conditions it would be warranted to investigate if a microbial community contains bacteria resistant to some specific antibiotic, for example given that there is an enrichment of total *E. coli*. In this context, simple indicators could be used to show when to study a particular environment in more detail, but it is clear that a defined set of criteria for when to make a more detailed risk assessment is urgently needed.

Currently, there are no standards for environmental monitoring of AMR in the same way as there are for monitoring in clinical, agricultural and food production settings. Given the various goals and many different and (potentially) non-overlapping methodologies currently applied in environmental AMR monitoring, it may be premature to implement such standards. A first step towards standardization would be to establish how the different methods used today compare to each other. Eventually, formal standardization for AMR monitoring in the environment should be a goal, but it is not at all clear what such a standard would look like. It is also reasonable to argue that relatively wealthy countries looking to implement AMR monitoring in the environment should support countries with less resources with the knowledge and technologies resulting from their implementation and operation of monitoring processes. From this perspective, the choice of methods should be based not only on what would be best in terms of scientific value, but also on what is feasible from, first and foremost, an economic perspective. It is also important that whatever standard is settled upon should incorporate targets that would be informative also in other settings and countries, which may have considerably less resources for AMR surveillance.

Similarly, the choice of locations and type of environments to monitor for AMR is also not entirely clear. A relevant starting point would be to utilize the already existing locations integrated into operating or planned surveillance programs. As mentioned earlier, surveillance is already performed for, among other things, drinking and bathing water quality, food safety and – more recently – sewage. Furthermore, AMR monitoring is already carried out in veterinary settings. Thus, integrating monitoring of AMR in the environment into existing monitoring infrastructure can be coordinated. Simply utilizing these monitoring points would already constitute a wide-reaching monitoring network and increase the amount of knowledge regarding AMR presence and transmission tremendously. Still, this would not be comprehensive enough. From a research point of view, more efforts should be made to identify if there are additional, less evaluated, pathways for AMR transfer via the environment, as well as if there are unexplored environmental settings where the selection of AMR and

evolution of novel ARGs take place. Such environments for transmission and development may, for example, be manure, wild animals (including birds) or urban parks, where very little investment has been made in monitoring.

11. Conclusions

We have here outlined a number of important knowledge gaps that impede the implementation of environmental AMR monitoring. Closing these knowledge gaps is crucial for a comprehensive understanding of the dissemination routes of resistant microorganisms outside of clinical and veterinary settings, of the settings in which AMR evolves and is selected for, and of human health risks in relation to environmental AMR. We are still not at a level of understanding where routine monitoring for AMR in the environment can be easily justified or implemented. That said, there is still a need for AMR monitoring data across different natural environments and including data from all sectors in order to fill these knowledge gaps. Hence, we support the implementation of national, regional and global initiatives without having all the scientific answers. With technology rapidly advancing and considering the urgency of the AMR issue, filling the important knowledge gaps will eventually happen, as will the development of standardized protocols. The lack of comprehensive understanding should not be an obstacle to starting environmental monitoring for AMR, nor for action against environmental development and spread of AMR. Ultimately, early adoption of monitoring will provide important puzzle pieces in the global and urgent efforts to combat AMR in order to safeguard human and animal health and wellbeing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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