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Towards monitoring the invisible threat: a global approach for tackling AMR in water resources and environment

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The global threat of antimicrobial resistance (AMR) is now increasingly recognized for the danger posed by its environmental spread. Aquatic environments and wastewater represent a significant diffusion and selection pathway for antibiotic resistance genes and antibiotic resistant bacteria (ARGs and ARBs). During a collaborative hackathon event, the "Innovation Workshop on Water Quality Monitoring & Assessment," held in September 2023, experts addressed four challenges related to water quality, including the challenge of globalization AMR surveillance in water. This paper, derived from the workshop findings, proposes a globally adaptable model for antimicrobial resistance surveillance intended as an advance to improve future monitoring systems. The new framework aims to address significant challenges, such as the lack of standardized methodological approaches or lack of funding, coordination, and awareness across a short-, medium- and long-term plan, integrating sustainability concepts, extending participation and monitoring capacity of countries, and offering efficient solutions. This vision is first articulated by creating a technical committee that promotes awareness of antimicrobial resistance and develops a single data management and communication platform. Subsequently, by developing local, national, and international policies, centralized laboratories will be established at the regional level, and built based on existing realities. These laboratories will include facilities to make the management of analyses more efficient, from sampling to reporting the final result. In the long term, activities that allow the maintenance of the created framework and continuous technological development and advancement will be promoted. All this will be achieved in collaboration with national and supranational bodies that are already addressing the issue at a global level.

KEYWORDS

antimicrobial resistance, surveillance, water, wastewater, water quality

Introduction

Antimicrobial resistance (AMR) refers to resistance developed by bacteria, viruses, fungi, and parasites, rendering them unresponsive to antimicrobial drugs (World Health Organization, 2014). This silent threat resulted in approximately 4.95 million deaths in 2019 associated with bacterial AMR (Murray et al., 2022). Projections indicated that this emerging threat could lead to 10 million deaths annually by 2050 (Review on Antimicrobial Resistance, 2016), accentuating the critical need to address this issue.

AMR, once seen as confined to clinical settings relating to only human and animal health, is now increasingly recognized for its environmental dissemination (Bengtsson-Palme et al., 2023; Hart et al., 2023). This has led to antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) being considered an emerging environmental pollutant of concern (Pruden et al., 2006; Gillings, 2018).

Water environments represent a significant pathway of AMR, facilitating its spread from natural water sources and wastewater (Liguori et al., 2022), including urban domestic sewage, agricultural runoff, and industrial discharges (Chu et al., 2018; Stanton et al., 2022). Consequently, water bodies can serve as reservoirs for ARB, ARGs, and mobile genetic elements (MGEs) (Kasuga et al., 2022), posing a potential health risk for humans and animals reliant on aquatic resources (Bengtsson-Palme et al., 2023). Therefore, monitoring and understanding AMR in various water environments is imperative for ensuring water safety.

Surveillance of AMR in natural and wastewater environments is crucial for multiple reasons. It helps understand how water and the broader environment contribute to the emergence and transmission of AMR across humans, animals, plants, and environmental niches (Liguori et al., 2022). Additionally, environmental surveillance provides crucial insights into the baseline of natural resistance, enabling the identification of changes in resistance patterns over time and the effectiveness of mitigation efforts (Bengtsson-Palme et al., 2023; Hart et al., 2023).

Surveillance in wastewater, due to its direct link to human populations and public health, offers valuable insights into the effectiveness of intervention strategies (Choi et al., 2018) and helps to identify potential risks, such as the persistence of ARB after water treatment, which could lead to potential infectious disease outbreaks (Manoharan et al., 2022). The recent SARS-CoV-2 pandemic demonstrated the value of wastewater as a crucial information source, providing aggregate samples representative of diverse sectors (Javvadi and Mohan, 2023). Furthermore, wastewater and wastewater treatment plants (WWTPs) are crucial environments for the potential selection of ARGs and ARB, given the presence of various contaminants that facilitate horizontal gene transfer (Partridge et al., 2018; Stanton et al., 2022). Consequently, WWTPs serve as both sources and reservoirs of AMR, underscoring the essential need for their surveillance (Karkman et al., 2018). Recognizing the interconnectedness emphasized by the recent pandemic, there is a pressing need for collective community and global actions to address these potential health threats (Cutrupi et al., 2022; Tegally et al., 2023).

To mitigate the threat posed by AMR, a One Health approach – encompassing humans, animals and the environment – is required (World Health Organization, 2014; European Commission, 2017; Djordjevic et al., 2023), yet many AMR national action plans still lack environmental components (Willemsen et al., 2022). It is essential to monitor resistance globally in environmental hotspots, where it develops and spreads. However, routine surveillance faces obstacles due to the complexity of its implementation in terms of coordination (lack or limitation) among countries and institutions, funding (lack at national and regional levels) and regulations/guidelines, and the absence of universally accepted standards on AMR in water resources (Berendonk et al., 2015; Bengtsson-Palme et al., 2023).

Under the flagship of the World Meteorological Organization (WMO), United Nations Environment Programme (UNEP), United Nations Educational, Scientific and Cultural Organization (UNESCO), World Water Quality Alliance (WWQA) and the European Commission's Joint Research Centre (EC-JRC), in cooperation with International Atomic Energy Agency (IAEA) and the United Nations Institute for Training and Research (UNITAR), a collaborative hackathon event "Innovation Workshop on Water Quality Monitoring & Assessment" was organized to tackle four challenges related to water quality ("Data to Action: Transforming data into actionable insights for water stewardship," "Empowering citizen scientists to improve water quality," "Melding AquaWatch & Global Indigenous Knowledge," "Routine monitoring of Antimicrobial Resistance), among which the challenge of global AMR surveillance in water. This paper, resulting from the hackathon event, proposes a globally adaptable model for AMR surveillance intended as an advancement to improve future monitoring systems. The new framework aims to address significant challenges in global AMR monitoring by integrating sustainability concepts, extending countries' participation and monitoring capacity, and offering efficient solutions.

Practical and methodological considerations

The clear need to implement antimicrobial resistance monitoring in natural environments and wastewater is challenged by many barriers related to the complexity of its implementation. A significant global barrier to effective antimicrobial resistance surveillance in water is the lack of standardized approaches (Liguori et al., 2022; Yin et al., 2023). Various methods exist to monitor antimicrobial resistance in the environment, including culturing ARBs, quantifying ARGs, and analyzing communities and genes using sequencing approaches such as amplicon sequencing, metagenomics, and metatranscriptomics (Stanton et al., 2022). Table 1 shows the pros and cons of the cited methods. However, bacterial culture-based methods present difficulties in addressing the multiple challenges of environmental antimicrobial resistance. Instead, methods that monitor ARGs using quantitative polymerase chain reaction (qPCR) or metagenomics are recommended (Miłobedzka et al., 2022). However, adopting such surveillance approaches could be challenging, especially because regulatory provisions on water and wastewater monitoring only sometimes incorporate molecular techniques. Furthermore, different environments may contain different pollutants and stressors, leading to differences in the potential gene targets for antimicrobial resistance surveillance (Keenum et al., 2022).

Setting up adequate routine monitoring, particularly on a regional scale, presents complexities due to factors such as budget, local characteristics (relating, for example, to the sampled environment),

TABLE 1 Relevant pros and cons of ARGs and ARBs detection methods.

Methods	Pros	Cons
Culture-based methods	Adaptable in environments with fewer resources. It allows for the prioritization of pathogens to search and gives information on phenotypic resistance, and which specific bacterium is carrying it.	Some bacteria, especially environmental ones, may be resistant to antibiotics but are not cultivable. Long and laborious method. Focusing only on some resistances does not allow a complete vision of the overall risk.
Quantifying ARGs through qPCR	Allows the detection and quantification also of rare ARGs. With some technologies, it is possible to target many ARGs simultaneously.	The selection of ARGs or other gene targets is carried out *a priori*. Not suitable for the discovery of new ARGs.
Metagenomics approaches	Ability to identify and quantify thousands of ARGs in a single sample. It can provide additional information on the presence of bacterial species, pathogens and virulence genes. The data can be reanalyzed if new genes of interest are identified.	Difficulty in identifying rare ARGs. Challenges in comparing metagenomic data due to the numerous ways to generate, analyze, and interpret the data. Need for specialized personnel to interpret the results. Difficult to apply in a low-resource area.

expertise available for sampling and analysis, logistics and objectives of the monitoring program (Behmel et al., 2016). The challenges become more significant in low-resource countries, with a lack of infrastructure and resources for sample analysis, preventing them from effectively monitoring and mitigating the growing threat of antimicrobial resistance (Africa CDC, 2023).

Many publications have suggested best practices for monitoring antimicrobial resistance in the environment. Table 2 shows some insights from international literature that can help to create an adaptable and scalable monitoring approach.

An adaptable global framework for monitoring AMR in water

Despite the abundant information present in the literature and the commitment provided by various bodies and entities at an international level, the challenge of establishing standardized monitoring that can be adapted in various global settings remains unresolved. A standardized approach is essential to ensure that data on AMR are consistent, reliable, and comparable across different regions. A standardized surveillance approach would fit into an adaptable global framework for routine water antimicrobial resistance monitoring. This surveillance must focus on the following:

1. The transmission and emergence of AMR in human populations and
2. AMR pollution in the environment.

Achieving the first objective requires monitoring AMR in wastewater due to its relation with the human community. In contrast, the second objective could be achieved by monitoring AMR in both natural and wastewater systems. This second objective could assess newly emerging resistance, create baseline data to inform future target limits of AMR levels and mitigation and aid in evaluating the efficacy of interventions. Sampling will consider the spatiotemporal variability of the water sampling point's physical, chemical and biological parameters. A preliminary characterization of the samples will be performed before the sampling campaigns intended to cover AMR. For instance, seasonal variations would be highly influenced by the geographical location and climatic conditions that need to be monitored (rainfall, temperature) as they may engender dilution/concentration (by evaporation). Hence, when required, more frequent sampling campaigns can be carried out. As per the sample type (grab or composite), guidelines and SOPs for water analysis should be used to guarantee QA/QC.

To address the surveillance of AMR in human populations, particularly focusing on the emergence of AMR, we propose regular monitoring of untreated wastewater monthly at a minimum, with a suggestion to undertake reactive, intensive sampling in response to local issues of concern. This wastewater-based monitoring approach can potentially provide insight into the distribution of AMR in the community and the potential source of resistance (Pruden et al., 2021). Additionally, analyzing spatial and temporal changes in AMR may provide an early warning of local and regional disease outbreaks (Prieto Riquelme et al., 2022). This routine monitoring plan can be used to complement the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) by monitoring raw wastewater at the same hospitals that are part of the GLASS network (World Health Organization, 2022). Following the One Health approach, a similar strategy can also be adopted in the animal health and agricultural domains, namely by monitoring wastewater from livestock farms.

To monitor aspects of AMR pollution in the environment, both natural and wastewater resources could be analyzed before and after interventions (e.g., changing wastewater treatment or improved farming practices) at least twice a year. However, the sampling analysis and frequency increases can be expected. This monitoring could provide a holistic understanding of the environmental dynamics and the potential of human activities on AMR dissemination. Environment protection agencies shall be involved in the monitoring plan. Their participation could contribute to developing effective and sustainable strategies for mitigating AMR pollution. At a broad scale, ESBL *E. coli* could be initially used as a target for surveillance, which would complement the ongoing WHO Tricycle project (World Health Organization, 2021). However, the monitoring scope can be expanded to include other specific AMR targets based on the local conditions and needs, such as different regional prescribing and usage trends or particular local pollution issues.

TABLE 2 Relevant publications on AMR and main primary outcomes.

Main results and suggestions	Authors
From a systematic literature review, a survey and an expert workshop, these are the main findings: - Design a framework addressing diverse global situations and research questions; - Developed guidelines for the conservation of samples to facilitate sharing among researchers and cooperating nations; - Water utilities should be involved in AMR monitoring through incentives or regulatory requirements.	Liguori et al. (2022)
Opinion article presenting key knowledge gaps, future research needs, policy and management options to be prioritized following the interdisciplinary and multidisciplinary COST Action project DARE (Detecting Evolutionary Hotspots of Antibiotic Resistance in Europe, TD 0803)(2009–2013), involving 20 European countries and 123 scientists. - Proposed a unique definition of resistance of environmental bacteria distinct from clinical pathogens; - Suggested the establishment of a standardized method, focusing on a subset of currently widely used resistance determinants and bacterial indicators; - Recommended specific ARB and ARGs as potential indicators for evaluating resistance status in environmental contexts.	Berendonk et al. (2015)
- Proposed four distinct objectives of AMR monitoring in the environment, which include: (1) transmissions of AMR, (2) acceleration risk of ARB evolution, (3) impact of AMR on ecosystem health, and (4) prevalence of AMR in the population; - Identified informative sites for monitoring.	Huijbers et al. (2019)
- Identified knowledge gaps of AMR in the environment, including the uncertainty in "normal" background levels of environmental AMR, concentrations of antibiotics and other resistance-inducing chemicals, and the lack of techniques for identifying resistance genes not presently circulating among pathogens. - Recommended incorporating objectives that offer information across diverse contexts and nations into any standard, with flexible options, especially regarding costs. - Emphasized the necessity to consistently update and integrate resistance data from non-clinical sources into ARB and ARG databases.	Bengtsson-Palme et al. (2023)
The results were reported from 150 publications containing data on ARGs, encompassing a total of 1,594 samples obtained from 12 distinct sample types across 30 countries. The key results included: - Certain matrices, such as those associated with human mobility and water linked to recreational activities, were under sampled in environmental monitoring; - Data on ARGs generated using qPCR should include absolute and relative abundances to facilitate comparisons across different studies. Lack of AMR information, particularly from Africa and South America.	Abramova et al. (2023)
- Suggested employing "ARG copy per cell" as a standard for reporting biological measurements in samples; - Enhanced comparability among surveillance efforts, addressing challenges posed by diverse analysis methods and approaches in bioinformatics analysis and facilitating the synthesis of results from multiple studies.	Yin et al. (2023)
The results of metagenomic analysis of bacterial resistance from 79 sites across 7 regions and 60 countries were reported. The key results included: - Systematic differences in AMR gene diversity and abundance between continents. - Correlation of AMR gene abundance with environmental, health, and socioeconomic factors. - Advocated wastewater metagenomics for global AMR monitoring.	Hendriksen et al. (2019)
The study reported results from the isolation of *E. coli* in eight hospital wastewater samples over one year in Gothenburg, Sweden, revealing that resistance data from sewage accurately reflected the resistance status in the studied populations. - Suggested calibrating sewage monitoring over time for an evolving clinical resistance situation. - Possibility of extending calibration from *E. coli* to other pathogens found in feces.	Hutinel et al. (2019)
- This work highlights how the environment, including environmental pollution from antibiotics, rather than the animal or human system, influences the evolution of resistance in bacteria and its transmission. - It also highlights how it would be necessary to study the use of antibiotics in agriculture, which to date has rarely been addressed despite the possible dangers arising, especially in low- and middle-income countries.	Larsson and Flach (2022)

A robust implementation plan to make the threat visible

To facilitate the implementation of routine monitoring of AMR globally, we recommend the creation of centralized laboratories at regional levels. These centers can be built upon existing facilities, like the Polio Laboratory Network (World Health Organization, 2017) or the AMR Surveillance CC Network (World Health Organization, 2023), and shall serve as hubs for the laboratory analysis of water samples, making the sample logistics efficient. The laboratories shall include facilities for sample handling, microbiological analysis, molecular biology analysis, and data science expertise. Regional hubs must follow standardized protocols for measuring ARBs and ARGs using microbiological and molecular methods. Still, they must be adaptable to different analytical methods and flexible to different geographical areas' specific antimicrobial resistance situations. The centers will conduct modeling analyses to determine specific AMR targets based on unique local and regional conditions. This approach considers the different geographical characteristics, the analytical capabilities and the sanitation and general health conditions that strongly impact the global AMR levels (Hendriksen et al., 2019), allowing the identification of precise objectives for monitoring. Until the set-up of regional centers is finalized, we encourage using culture-based methods for AMR monitoring in water at the local level following the WHO Tricycle protocol (World Health Organization, 2021).

To implement the adaptable global framework, addressing the challenges posed by lack of funding, coordination, and awareness is necessary through a short, medium, and long term plan. Our proposal is summarized in Figure 1.

In the short term (1–3 years), the focus is on Management. We recommend setting up a Technical Committee, which should include different representative stakeholders from academia, the private sector, the public sector, international organizations, authorities at the local, regional, national, and supranational levels and non-governmental organizations (NGOs). This Technical Committee will complement existing committees and structures on an international scale, such as the European Public Health Wastewater Observatory or the Global Health Observatory (GHO) established by WHO. The Committee will have thematic subcommittees that will intervene in the interdisciplinary and transdisciplinary areas of the problem. After evaluating the existing data on AMR in the environment, the Technical Committee shall work with experts to prepare a communication campaign to raise awareness using diverse outreach materials and channels. The cost of inaction should be clearly communicated to stakeholders. Social science experts should also be consulted to increase public engagement through storytelling and personification of the problem, to communicate the urgency of the AMR issue and the fact that it also affects individuals on a personal level. The increased awareness should facilitate coordination and help fundraising from partnerships, donors, governments and the private sector.

In addition, we propose the creation of a "Smart Interactive Platform for AMR management," serving as a unique data management and communication platform for all involved stakeholders. A pilot version of this platform could be created in collaboration with other ongoing international initiatives focused on water quality. Otherwise, the Technical Committee could follow the example of other international agencies that outsource the construction of similar platforms with standardized monitoring and data management methods. AMR monitoring data shall be continuously added to the platform with other relevant water quality parameters. Uploaded data shall be used to increase awareness of the AMR issue further. The Technical Committee shall coordinate the set-up of the AMR platform, the definition of roles, and the evaluation of the outcomes of each activity. While maintaining supranational coordination, country-level representatives and focal points shall be appointed.

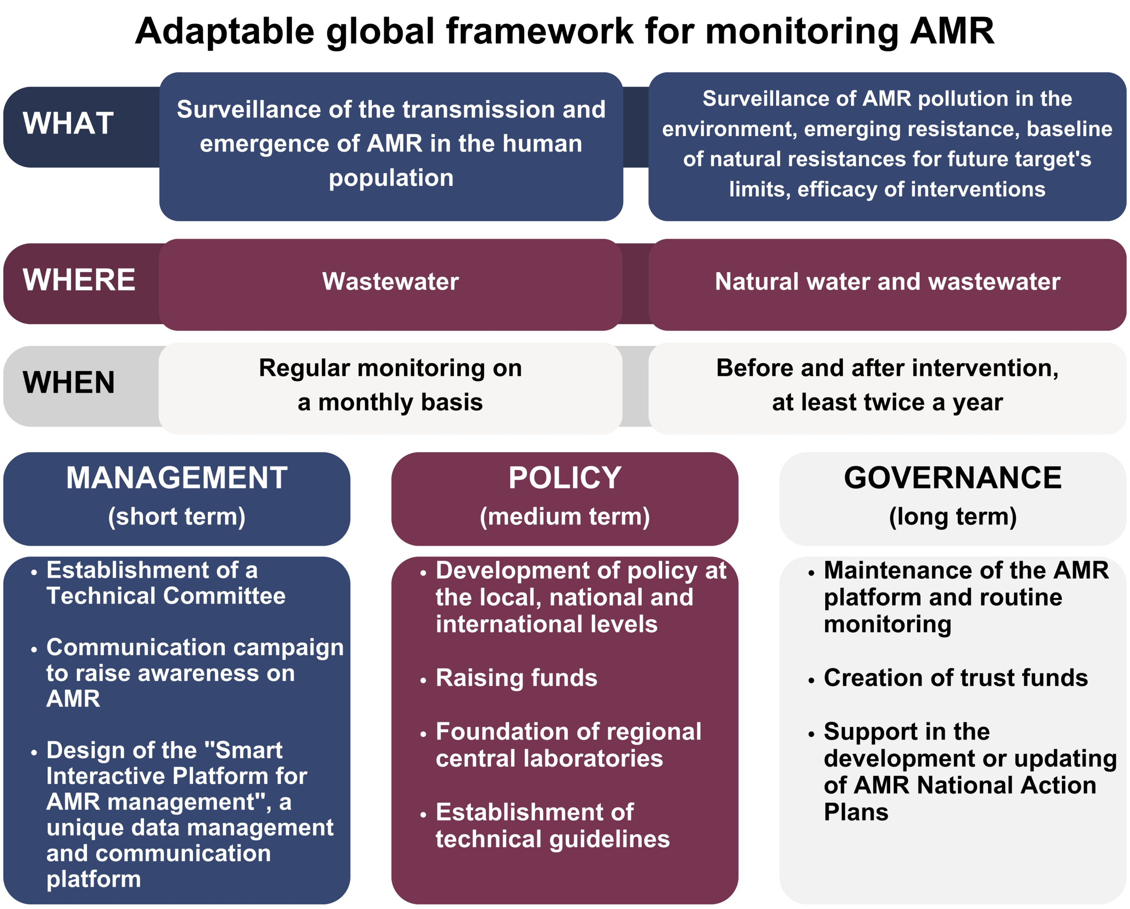

FIGURE 1
Adaptable global framework for routine monitoring of AMR in water and wastewater.

In the medium term (3–5 years), the focus is on Policy. The increased communication and awareness provided by the Smart AMR platform shall aid the development of local, national, and international policies and raise funds, promoting the routine monitoring of AMR through the proposed adaptable global framework. An example of a related policy is the European Commission's proposal for a revised Urban Wastewater Treatment Directive, which introduced a provision that, if approved, would require the European Union Member States to monitor AMR at least twice a year at the inlets and outlets of urban wastewater treatment plants serving larger agglomerations (European Commission, 2022). Regional central laboratories can be funded through either one of the following business model options: subscription per country; each country pays a fixed fee per sample; or fundraising from donors or international organizations. Fundraising strategies can be similar to those of other organizations: through communication channels (conferences, high-level meetings, etc.), representatives of the Technical Committee will present the fundraising plan to potential donors. This plan will include low-income countries ready to have central regional structures (regarding national interest, available human resources, etc.). Having a centralized organization will allow for cross-funding from funding agencies to maintain the continuity of the Laboratory. In this phase, the Technical Committee shall be replaced by AMR Communities. The AMR Communities will be more localized for operational efficiency. This comprises NGOs, Research Institutions, and government agencies tasked with grassroots or community responsibilities. Their role will be to support the coordinator of local monitoring and relay information to the central regional laboratory or the representative units, ensuring the feasibility of monitoring activities. The availability of more AMR monitoring data will push the creation of technical guidelines with the support of the centralized regional laboratories. Activities shall be subject to periodic evaluation, for example, by other laboratories focusing on water quality or by ISO certification bodies, and to measuring key performance indicators (e.g., number of samples analyzed, number of countries involved) to ensure their fitness for purpose.

In the long term (5–10 years), the focus is on Governance. Activities shall include the maintenance of the AMR platform, capacity building, education and training, technology advancement and transfer, creation of trust funds, and development of action plans. The global framework for routine AMR monitoring should be implemented into AMR National Action Plans.

Considerations on property rights, data ownership and data sharing are challenges that need further discussion and addressing. Legal experts on data governance should be involved in this discussion, with consideration for the Nagoya Protocol (Convention on Biological Diversity, 2014). Additional challenges for implementing the framework are the translation of technology, data elaboration, and sustainability regarding the project's continuity. Continuity can be ensured through sufficient funding and commitment of the involved stakeholders.

Conclusion

The issue of AMR is vast and complex. Some aspects need to be explored further, such as the natural resistance levels in the environment or all the factors, anthropic or otherwise, that contribute to the selection of ARGs and ARBs. Indeed, the winning approach involves surveillance that is as global and shared as possible, including each Country's contribution and support from the wealthier Nations toward the States that present more significant difficulties. In such conditions, the chosen approach must be flexible and adaptable to the different regional conditions, which may vary due to many factors, such as the use of different types of antibiotics, the quality of infrastructure, the presence of various pollution levels and the socio-economic conditions of the monitored area. Last but not least, surveillance cannot stop at the human sphere alone, as AMR is an environmental problem requiring a One Health strategy to be addressed adequately.

For these reasons and to counteract the difficulties mentioned and encourage the creation of a framework adaptable to different global conditions, the approach we suggest includes a short-, medium- and long-term vision. This vision is articulated first by creating a Technical Committee that promotes awareness of the AMR issue and develops a single data management and communication platform. Subsequently, by developing policies at local, national and international levels, centralized laboratories will be founded based on existing realities at regional levels. These laboratories will include facilities to make analysis management more efficient, from sampling to communicating the final result. In the long term, activities that allow the maintenance of the created framework and continuous technological development and advancement will be promoted. All this will be created in collaboration with national and supranational bodies that are already addressing the issue on a global level.

This article aims to contribute to the long road still to be covered in monitoring and evaluating the extent of antimicrobial resistance in treated and untreated water bodies and their impact on health and the environment. Standardized monitoring of AMR in aquatic environments would contribute to a comprehensive understanding of its prevalence and enable coordinated efforts to address this global threat. Overall, revealing global antimicrobial resistance in aquatic environments is critical to protecting human and animal health, preserving the environment, preventing the spread of resistance, ensuring water safety and taking a holistic approach toward combating antimicrobial resistance.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

FC: Writing – original draft, Writing – review & editing, Investigation. AO: Conceptualization, Writing – original draft, Writing – review & editing. IR: Writing – original draft, Writing – review & editing, Investigation. JA: Writing – original draft, Writing – review & editing, Investigation. YV: Writing – review & editing. OM: Writing – review & editing. JC: Writing – review & editing. DP: Conceptualization, Supervision, Writing – review & editing. WM: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

DP and WM are employees of Resistomap Oy. WM owns stock in Resistomap Oy.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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