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Towards Enhanced AMR Monitoring under the Recast Urban Wastewater Treatment Directive (EU) 2024/3019

A Stocktaking Review of EU Member States' Studies and a Proposal Outline for a Quantitative PCR-based Monitoring

2025

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Abstract

Antimicrobial resistance (AMR) is an escalating environmental and public health challenge, increasingly associated with the acquisition and dissemination of antibiotic resistance genes (ARGs) through urban wastewater systems. In response, the recast Urban Wastewater Treatment Directive (EU) 2024/3019 (UWWTD) mandates the establishment of harmonised minimum sampling frequencies and standardised methodologies for AMR monitoring across EU Member States. Specifically, UWWTD requires monitoring for AMR at those UWWTP's serving agglomerations of 100,000 population equivalents (p.e.) and above (currently numbering 1,287 UWWTPs across the EU, based on latest reporting).

This report reviews current AMR monitoring practices in urban wastewater across EU Member States to enhance data harmonisation and cross-national comparability, thereby improving the characterisation of AMR occurrence and significance in urban wastewater systems. Contributions from 12 Member States, encompassing 109 references, were analysed, revealing substantial heterogeneity in sampling designs and analytical techniques.
Culture-based methods remain prevalent, yet molecular approaches, particularly quantitative PCR (qPCR) and digital droplet PCR (ddPCR), are widely adopted due to their high sensitivity and specificity for detecting targeted ARGs. A total of 189 ARG targets were identified across 17 water matrices, highlighting the complexity and diversity of AMR profiles in water-based environmental matrices. Building on this evidence, this report proposes a targeted qPCR-based approach as the preferred methodology for routine AMR surveillance in urban wastewater. Compared to other potential methods, such as metagenomic analysis which provides broad resistome insights but remains resource-intensive, qPCR offers a cost-effective, scalable, and technically accessible solution, making it highly suitable for harmonised implementation across EU Member States.

A panel of 24 ARGs + 1 additional genetic target (*intI1*) in urban wastewater was defined through a structured process combining: (i) consultation with EU Member States and analysis of the references they submitted; (ii) review of ongoing stardardisation activities (CEN, ISO, ASTM) and of reports prepared by relevant expert networks (WHO, Eionet); (ii) input provided by JRC scientists and research partners; and (iv) a comprehensive assessment of the scientific literature.
The final selection was cross-checked against WHO List of Medically Important Antimicrobials (MIA List), the WHO Model List of Essential Medicines (EML List), and the WHO Bacterial Priority Pathogens List 2024 (2024 BPPL), as well as environmental AMR monitoring studies (such as the *Pilot study on antimicrobial resistance* (April, 2025) from the Eionet Working Group, ensuring relevance from both public health and environmental perspectives. The resulting panel reflects a balanced representation of clinically important and environmentally traceable resistance determinants, ensuring that the most relevant aspects of AMR are captured through a One-Health aligned approach, while acknowledging that the majority are antibiotic resistance genes – an emphasis justified by their central role in human health and environmental dissemination.
The suggested monitoring design foresees monthly sampling of both influent and effluent at urban wastewater treatment plants (UWWTPs), corresponding to 24 samples per facility per year. This frequency is designed to capture seasonal fluctuations in population behaviours, antimicrobial usage and patterns influencing the occurrence of AMR, while not entailing excessive costs.
The recast Urban Wastewater Treatment Directive (UWWTD), requires monitoring for AMR at those UWWTPs serving agglomerations of 100,000 population equivalents (p.e.) and above (currently numbering 1,287 UWWTPs across the EU, based on latest reporting).
This report further recognises that AMR monitoring in environmental matrices is a rapidly evolving

field. Important gaps remain, particularly concerning resistance mechanisms beyond antibiotics, which may have significant implications for One Health.

To address these uncertainties, this report recommends further research and validation of additional AMR determinants, supported by other potential methods such as metagenomic sequencing, here considered as a voluntary complementary tool. These approaches can capture the broader resistome, detect emerging genes and inform the progressive refinement of the proposed qPCR panel.

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This document was reviewed in its final stages with the assistance of Grammarly®. The feedback provided by Grammarly® was carefully evaluated and used as a basis for revising the writing. All revisions were made using the authors own words and expressions to ensure originality and authenticity.

It is important to note, though, that despite the assistance from these AI resources, the core content and texts within these proceedings are the result of genuine human intellectual efforts.

Policy context

The establishment of comprehensive surveillance frameworks under the One Health approach is crucial for enhancing public health protection, enabling more effective monitoring and ensuring timely response to health threats in an increasingly interconnected world. Urban wastewater is identified as of great significance in understanding antimicrobial agents and the circulation of resistant bacteria in the underlying population[1]. Urban wastewater, originating from domestic, hospital, industrial and other sources, is typically collected and treated in urban WWTPs, serving as both a reservoir and transmission pathway for antibiotic resistance genes (ARGs). Discharges of treated wastewater effluent and sewage sludge into the environment contribute to the spread of AMR through multiple exposure routes.

The European Union recognises the importance of tackling the issue of AMR, as stated in the 2017 Communication from the Commission 'A European One Health Action Plan against Antimicrobial Resistance (AMR)', and the adopted European One Health Action Plan against AMR. With the 2023 Council Recommendation on Stepping up EU Actions to combat AMR in a One Health approach Member States are encouraged to develop integrated systems for the surveillance of AMR, including in wastewater. The Recommendation recognises the role played by the presence in the environment of antimicrobial residues in the emergence and spread of AMR, the levels of environmental contamination and the risks posed to human health.

The recast Urban Wastewater Treatment Directive (EU) 2024/3019[2] recognises the interconnectedness of human, animal, and environmental health through the One Health approach and addresses the growing concern of AMR in urban wastewater. Article 17(3) of the Directive empowers the European Commission to adopt implementing acts in order to establish a minimum frequency of sampling and a harmonised methodology for measuring AMR in urban wastewater. The Commission is to adopt these implementing acts by July 2nd, 2026. Meanwhile, Article 22(1)(h) of the Directive requires Member States to report AMR monitoring results to the Commission by December 31st, 2030, with annual updates thereafter. Articles 17(1) and 17(2) of the Directive require Member States to set up national systems for cooperation and coordination between competent authorities for public health and urban wastewater treatment, facilitating early detection of public health parameters including SARS-CoV-2, polio, emerging pathogens and other health determinants deemed relevant by the competent authorities.

Joint Research Centre (JRC)'s effort has been to review literature on the topic, analyse Member States submitted references, and to appraise standardisation activities undertaken by international bodies such as the European Committee for Standardization (CEN), the International Organization for Standardization (ISO), and the American Society for Testing and Materials (ASTM).

[1] La Rosa MC, Maugeri A, Favara G, La Mastra C, Magnano San Lio R, Barchitta M, et al. The Impact of Wastewater on Antimicrobial Resistance: A Scoping Review of Transmission Pathways and Contributing Factors. Antibiotics. 2025 Jan 26;14(2):131.

[2] European Parliament & Council. (2024). *Directive (EU) 2024/3019 of 27 November 2024 on urban wastewater treatment (recast) (Text with EEA relevance)* (PE/85/2024/REV/1). https://eur-lex.europa.eu

Key conclusions

The review of literature and data from 12 Member States revealed substantial heterogeneity in sampling designs and analytical techniques for AMR monitoring in urban wastewater, highlighting the urgent need for standardised methodologies. While culture-based methods remain common, molecular approaches, particularly quantitative PCR (qPCR) and digital droplet PCR (ddPCR), are widely adopted due to their high sensitivity and specificity and cost/effectiveness for detecting targeted antibiotic resistance genes (ARGs). Nevertheless, targeted molecular approaches leave room for advanced techniques such as metagenomic sequencing, which can enhance understanding of the broader environmental resistome and emerging resistance mechanisms. The proposed qPCR monitoring framework facilitates the collection of standardised data and metadata, providing a solid foundation for understanding AMR dynamics and supporting surveillance aligned with One Health objectives.

Next steps in support of the implementing acts under the recast UWWTD include:

1. Experimental evaluation, optimisation and validation of the proposed analytical methods
2. Consultation with expert stakeholders and Member States active in AMR monitoring.
3. JRC providing guidance and evidence to the drafting of the implementing acts.

Structure of the report

This document has been structured to provide a comprehensive and cohesive analysis of the methodologies and challenges related to AMR monitoring. The sections are as follows:

1. **Introduction:** Sets the stage for the entire report, explaining the significance of monitoring AMR in urban wastewater systems. It introduces key concepts such as ARGs and highlights the importance of standardised methodologies. This section provides the rationale for the subsequent detailed examination of European level approaches.

2. **Methods:** This chapter outlines the data collection procedure and analysis. Between January 31st and February 28th, 2025, a call for contributions on AMR monitoring in urban wastewater was circulated to EU Member States and relevant networks. 109 contributions were received. Additional input from CEN, ISO, and ASTM was included. The review addressed only antibiotic resistance, reflecting the position of participating Member States.

3. **Findings:** This chapter presents the main outcomes of the review. It is organised into four interrelated subchapters, each focusing on a key aspect of AMR monitoring in urban wastewater. Data is summarised in Annex 1:

   - **Sampling and Analytical Methods**: This section examines the sampling strategies and analytical techniques reported in the literature shared by the different countries and currently in use to monitor AMR. It highlights the diversity of approaches in practice and reinforces the need for harmonised and robust monitoring strategies.

   - **ARGs Monitored**: Building on the previous section, this part details the specific ARGs identified across different monitoring efforts. It provides insight into how methodological choices are often influenced by the relevance and prevalence of particular ARGs at the European level.

   - **Standardisation**: Following the discussion on methodologies and detection outcomes, this section addresses the issue of standardisation. It explores national approaches to protocol development and highlights existing inconsistencies, highlighting the need for improved comparability of data across countries to support global AMR monitoring objectives.

   - **Descriptors for Monitoring**: This chapter focuses on the specific metrics and parameters used to quantify and interpret AMR-related data. It provides detailed insights into how data is quantified and later interpreted. It complements the standardisation discussion by stressing the importance of consistent descriptors to ensure meaningful, comparable results across monitoring systems.

4. **Other AMR monitoring initiatives**: The section highlights important global and regional initiatives, such as the World Health Organization (WHO) integrated global surveillance protocol for ESBL-producing *Escherichia coli* (ESBL-Ec), EU-Wastewater Integrated Surveillance for Public Health (EU-WISH), the European Environment Information and Observation Network (Eionet) working group and the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR). These, alongside the Member State contributions, help to render the picture on the collaborative efforts among countries to standardise AMR monitoring.

5. **Conclusion:** Synthesises the information presented in the report, reiterating the importance of standardised approaches and the diverse methodologies employed. It proposes concrete steps for wastewater-based AMR monitoring in Member States and raises additional aspects to consider when undertaking such activities. Overall, it ties together the significance of monitoring AMR, the challenges posed by diverse methods, and the necessity for harmonised protocols.

1. Introduction

Antimicrobial resistance (AMR) is recognised as one of the most pressing global threats to public health and environmental sustainability [3,4]. Antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) circulate across humans, animals, and the environment, undermining the effectiveness of treatments for infectious diseases[5]. Increasing evidence highlights that natural and treated water environment, play a critical role in the emergence, evolution, and dissemination of AMR [6].

Urban wastewater treatment plants (UWWTPs) receive influent from diverse sources, including households, hospitals, industries, and urban runoff. As such, they represent important pathways and reservoirs for ARGs, and in some cases potential hotspots for the amplification and dissemination of resistance determinants into the wider environment. Monitoring AMR in these systems is therefore essential to characterise risks, inform public health and environmental policies, and support targeted interventions aimed at mitigating the spread of resistance.

The recast Urban Wastewater Treatment Directive (EU) 2024/3019 (UWWTD) establishes a legal framework for tackling this challenge. Specifically, Article 17(3) mandates the development of a harmonised methodology and minimum sampling frequency for AMR monitoring in urban wastewater, while Article 22 requires Member States to report AMR datasets to the European Commission by 2030 and annually thereafter [7,8]. To support this implementation, the European Commission's Joint Research Centre (JRC) is providing scientific and technical input for the design, evaluation, and validation of AMR monitoring approaches.

This report reviews current AMR monitoring practices across EU Member States, drawing on contributions from literature and national initiatives. It examines sampling strategies and analytical techniques applied to detect and quantify ARGs in wastewater, with a focus on culture-based and molecular methods such as quantitative PCR (qPCR), digital droplet PCR (ddPCR), and metagenomics. A key emphasis is placed on the need for harmonised methodologies and metadata collection, to ensure comparability of results across countries and support One Health-aligned surveillance objectives.

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[3] Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. The Lancet. 2024 Sep 28;404(10459):1199–226.

[4] Ajulo S, Awosile B. Global antimicrobial resistance and use surveillance system (GLASS 2022): Investigating the relationship between antimicrobial resistance and antimicrobial consumption data across the participating countries. PloS One. 2024;19(2):e0297921.

[5] CDC. Antimicrobial Resistance. 2025 [cited 2025 Apr 9]. Antimicrobial Resistance Threats in the United States, 2021-2022. Available from: https://www.cdc.gov/antimicrobial-resistance/data-research/threats/update-2022.html

[6] US EPA O. National Priorities Grants: Evaluation of Antimicrobial Resistance in Wastewater and Sewage Sludge Treatment and Its Impact on the Environment [Internet]. 2024 [cited 2025 Jul 22]. Available from: https://www.epa.gov/research-grants/national-priorities-grants-evaluation-antimicrobial-resistance-wastewater-and

[7] Manaia CM. Framework for establishing regulatory guidelines to control antibiotic resistance in treated effluents. Crit Rev Environ Sci Technol. 2023 Mar 19;53(6):754–79.

[8] European Commission. Impact Assessment accompanying the Commission proposal for a recast UWWTD [Internet]. Brussels: European Commission; 2022 Oct [cited 2025 Jul 22]. Report No.: SWD(2022) 541 final. Available from: https://circabc.europa.eu/ui/group/65764c73-4a57-45dc-8199-473014cf65bf/library/889da570-1fb0-479c-bc7c-3104ad7c8feb/details

By consolidating existing knowledge and practices employed in different EU countries, this report provides the scientific basis for developing standardised AMR monitoring protocols under the recast UWWTD. These efforts will be instrumental in advancing evidence-based policy, fostering international alignment, and ultimately contributing to the containment of AMR in Europe's urban environments.

2. Methods

On 31 January 2025, an invitation to participate in this review was sent to all EU Member States. National authorities were asked to forward the call to competent organisations within their countries. The request was additionally disseminated via the EU-Joint Action Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI), the EU-WISH Joint Action (JAs), and the Health Security Committee (HSC).
Potential contributors were invited to submit relevant studies and reports on antimicrobial resistance (AMR) monitoring in urban wastewater. The call for contributions remained open until 28 February 2025.

All submitted documents were reviewed with the support of the GPT@JRC tool, which integrates large language models (Llama 3.3 70b instruct and GPT-4o). An ad hoc prompt template (Annex 1) was applied to extract and classify information.
Specifically, the review focused on (i) sampling methods used for wastewater monitoring, (ii) analytical methods applied for AMR/ARG detection, (iii) antibiotic resistance genes (ARGs) and other targets monitored, (iv) descriptors relevant for harmonisation, (v) standardisation efforts reported at national or international level.

In parallel to the Member State submissions, the JRC conducted a desk-based review of standardisation initiatives relevant to AMR monitoring in wastewater. Efforts by international organisations, including the European Committee for Standardisation (CEN), the International Organization for Standardization (ISO), and ASTM International, were considered. The extracted information was systematically collated and analysed. Descriptive statistics and visualisations were produced using the R statistical environment.

It is important to note that the contributions received provide expert insights from Member States on AMR monitoring. While they do not necessarily reflect current national practices, they offer valuable perspectives on the state of the art and contribute to identifying best practices and future directions for implementation.
Furthermore, while the recast UWWTD defines AMR broadly as the ability of microorganisms (bacteria, viruses, fungi, parasites) to survive or grow in the presence of antimicrobial agents (Article 2(22)), the survey responses and received literature from Member States, focused primarily on antibiotic resistance. This emphasis reflects both the state of the scientific evidence and the public health relevance of antibiotics. At present, antibiotic resistance is the most extensively documented component of AMR in wastewater systems, supported by molecular detection methods and standardisation activities at international level. In contrast, systematic monitoring frameworks for antiviral, antifungal, or antiparasitic resistance remain largely undeveloped, with limited data available in the peer-reviewed literature. Consequently, antibiotic resistance serves as a scientifically robust and policy-relevant proxy for AMR in wastewater, while recognising the need for future research to extend monitoring approaches to other microbial classes.

3. Findings

A total of 109 literature sources, including peer-reviewed papers, country-specific activity reports, and project summaries, were received. Information extracted from the literature contributions shared by each Member State are summarised in Annex 2.

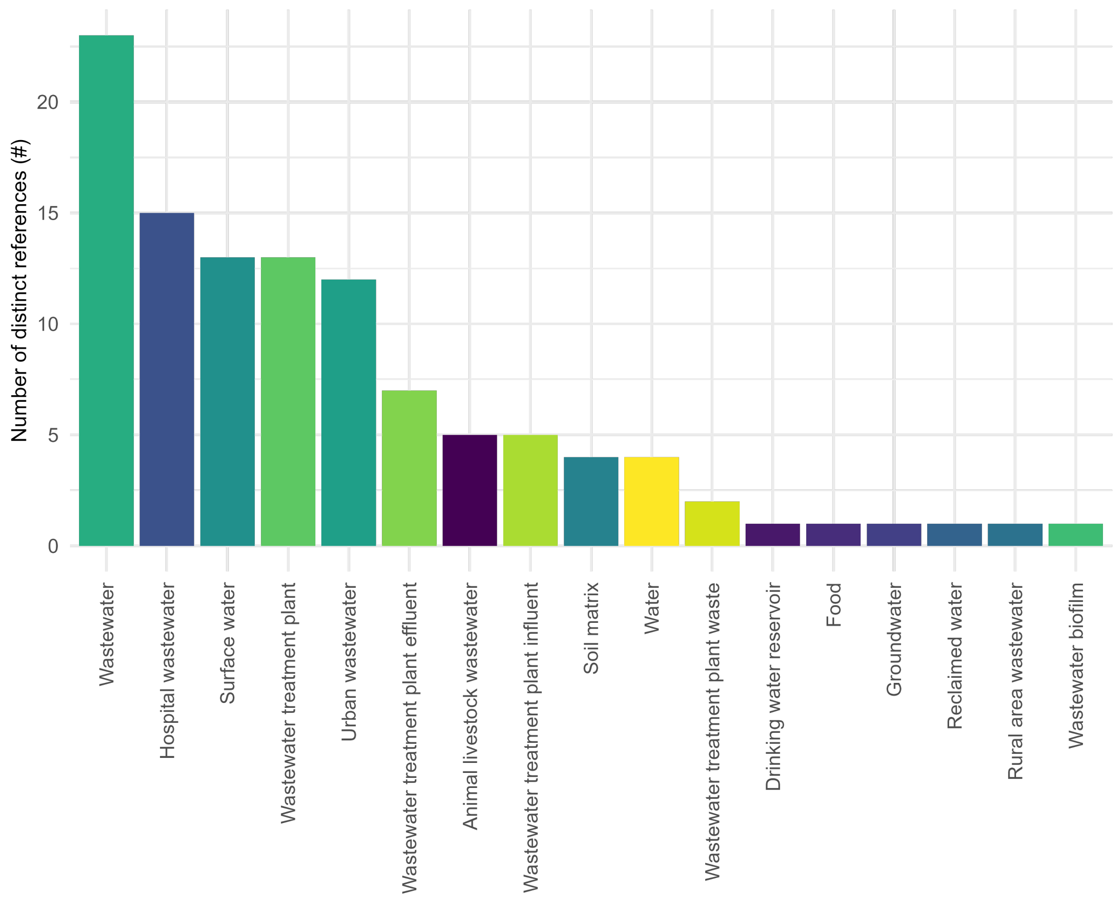
Sampling and analytical methods

The documentation provided by Member States reveals that a variety of sampling and analytical methods are currently applied for AMR monitoring in wastewater. These practices are adapted to the specific objectives of the implementing institutions and reflect both scientific and operational considerations.

Sampling methods. Composite sampling conducted over a 24-hour period was reported twice as often as grab sampling. In total, 12 Member States reported the use of one or both approaches, with several indicating that composite and grab samples are applied in combination depending on study design and available infrastructure. Composite sampling provides time-averaged information that captures daily variability, whereas grab sampling is more suited to targeted investigations of specific events or localised sources. Some countries, including Austria, Belgium, Sweden, and Portugal, reported more refined composite sampling strategies, applying flow-, time-, or volume-proportional collection. Autosamplers were most frequently reported for composite sampling, improving representativeness, while minimising manual labour and contact with wastewater.

Monitored matrices. Wastewater was the principal environmental matrix studied for AMR monitoring, with 23 studies explicitly reporting on this source. Several studies included multiple wastewater sources within a single investigation, for instance combining domestic and hospital effluents. Hospital wastewater was analysed in 15 studies, UWWTP influent in 13 studies, and UWWTP effluent in 12 studies. In most cases, both influent and effluent were sampled within the same study, allowing assessment of treatment performance as well as temporal and spatial variation in ARG abundance across the wastewater system. Although less frequent, other matrices such as agricultural run-off, farming effluents, and surface waters were also included. Surface water was the third most common matrix overall (n = 13) (Figure 1), reflecting its importance as a receptor environment for UWWTP discharges.

Figure 1. Number of distinct references citing different water-based environmental matrices



Source: EC-JRC.

Analytical methods. Culture-based assays were still widely reported, often serving as a first step prior to molecular analysis or sequencing. These were complemented by PCR-based methods, including qPCR, ddPCR, and HT-qPCR, which were highlighted in submissions from Austria, Belgium, Finland, France, Germany, Italy, Netherlands, Portugal, and Sweden. Digital PCR approaches (mainly ddPCR) were reported by Austria, Belgium, Finland, Germany, Italy, and Portugal. Whole-genome sequencing of bacterial isolates following culturing was less common but still present in several references. More advanced approaches included metagenomics, particularly shotgun sequencing, reported by Austria, Belgium, Bulgaria, Finland, the Netherlands, Portugal, and Sweden. Additional methods such as 16S rRNA gene sequencing (for microbial community profiling), metatranscriptomics (focussing on ARG expression), hybrid capture-based sequencing, and standard phenotypic susceptibility testing (e.g. Kirby–Bauer disk diffusion) were also reported, albeit less frequently.

Literature shared by Member States does not necessarily document national practices in a systematic way, but rather reflects studies deemed most relevant to this work at the time of the request. The review provides valuable insight into the current landscape of methodologies, but it is not a comprehensive mapping of operational practices across each Member State.

ARGs monitored

The analysis of submissions by EU Member States identified 109 references reporting on 189 distinct ARG targets across a range of water-based environmental matrices. These matrices included urban and hospital wastewater, surface waters, soil, and wastewater biofilms. Of these, 24 ARG targets were specifically monitored in urban wastewater (Table 1; Figure 2). The widest diversity of ARGs was documented in soils, surface waters, hospital effluents, and biofilms, emphasising the multiplicity of environmental compartments contributing to ARG occurrence. It is important to note that the identification of these 189 ARGs was based on single-gene targeted molecular techniques (e.g., qPCR, ddPCR), reflecting the predominant reliance on such assays in current monitoring practice.A complete list of all ARGs reported by Member States is provided in Annex 2.

Among them, the 24 most frequently cited ARGs + 1 additional genetic target (intl1) in urban wastewater (Table 1,2) were identified according to the number of references in which they were reported. While some of these ARGs were also detected in other environmental matrices (such as surface waters or hospital wastewater), their recurring detection in urban wastewater highlights the pivotal role of this matrix as both a reservoir and a dissemination pathway for ARGs into the aquatic environment.

Table 1. Most frequently cited ARG targets (n. 25) in urban wastewater (ranked by number of citations).

N.	ARG target	# of citations	N.	ARG target	# of citations
1	*blaCTX-M*	55	**13**	*blaIMP*	15
2	*blaOXA*	51	**14**	*blaSHV*	15
3	*blaNDM*	37	**15**	*sul2*	15
4	*sul1*	35	**16**	*blaCMY*	14
5	*blaKPC*	30	**17**	*qnrS*	13
6	*blaTEM*	29	**18**	*blaGES*	12
7	*blaVIM*	26	**19**	*mecA*	12
8	*tetA*	25	**20**	*cmlA*	11
9	*vanA*	25	**21**	*dfrA*	11
10	*mcr-1*	23	**22**	*tetW*	11
11	*ermB*	22	**23**	*aac(6')-Ib*	10
12	*tetM*	20	**24**	*aadA1*	10

Source: EC-JRC

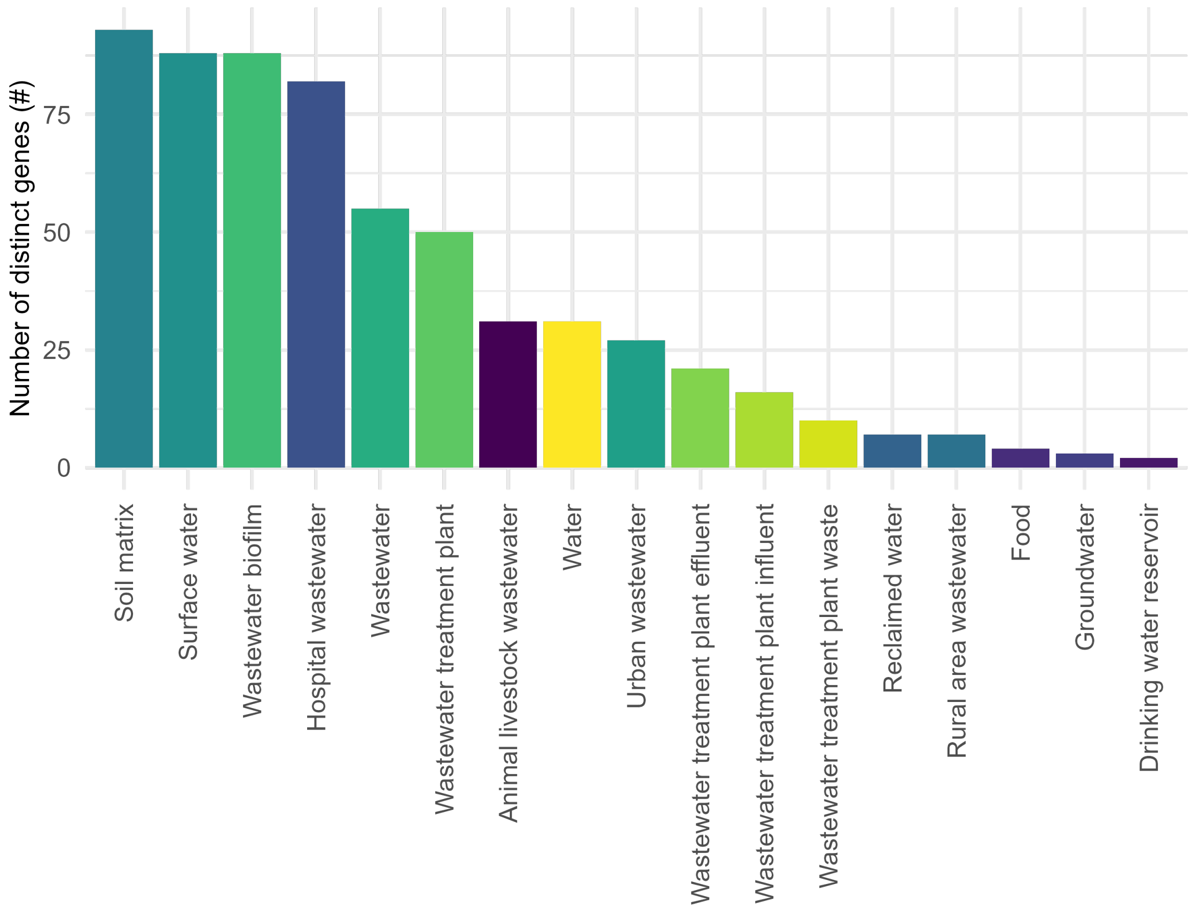
In addition to ARGs, the class 1 integron-integrase gene (intl1) was also frequently reported (Table 2). While not an ARG itself, intl1 is widely recognised as a proxy for horizontal gene transfer (HGT) and as an indicator of the potential for ARG mobilization and dissemination [9].

Table 2. Additional genetic target reported and number of citations. *Source: EC-JRC*

N.	Target	# of citations
25	*intl1*[1]	16

[9] Ghaly TM, Gillings MR, Penesyan A, Qi Q, Rajabal V, Tetu SG. The Natural History of Integrons. Microorganisms. 2021 Nov;9(11):2212.

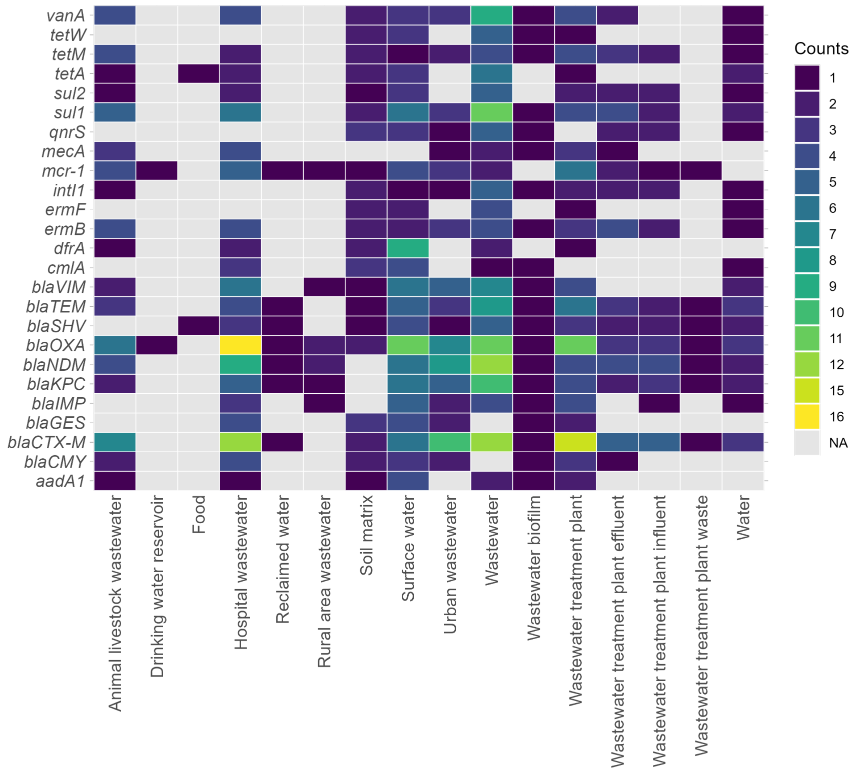
Figure 2. Number of distinct ARGs identified across environmental matrices. Visualisation and performed in R (https://www.R-project.org/).



Source: EC-JRC

Analysis of ARGs across environmental matrices shows that blaCTX-M was the most frequently studied gene in wastewater, followed by blaOXA, blaNDM, sul1, blaKPC, blaTEM, blaVIM, tetA, vanA and mcr-1 (Figure 3).

Figure 3. Heatmap of the 25 most frequently cited ARG targets across environmental matrices. Visualisation performed in R (https://www.R-project.org/).



Source EC-JRC

The *blaCTX-M, blaOXA*, *blaNDM, blaKPC, blaTEM* and *blaVIM* families have received greatest attention due to their central role in conferring resistance to extended-spectrum beta-lactam (ESBL) antibiotics, including penicillin, cephalosporins and carbapenemases. These genes were among the most frequently identified in urban wastewater, highlighting their critical role in antimicrobial resistance (AMR). The resistance they confer is of highest concern globally:

- ESBLs are classified under all priority categories of the WHO List of Medically Important Antimicrobials (MIA List)[10], the WHO Model List of Essential Medicines (EML List)[11], and the WHO Bacterial Priority Pathogens List 2024 (2024 BPPL)[12].

- In the 2024 BPPL, carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Enterobacterales* and third-generation cephalosporin-resistant *Enterobacterales* are identified as top global health threats, due to limited treatment options and few alternative therapies in development.

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[10] WHO List of Medically Important Antimicrobials: A risk management tool for mitigating antimicrobial resistance due to non-human use. https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf

[11] WHO Model List of Essential Medicines – 23rd List, 2023. https://iris.who.int/bitstream/handle/10665/371090/WHO-MHP-HPS-EML-2023.02-eng.pdf?sequence=1

[12] WHO Bacterial Priority Pathogens List, 2024: https://iris.who.int/bitstream/handle/10665/376776/9789240093461-eng.pdf?sequence=1

The *sul1* gene also ranked highly, reflecting both its widespread detection in wastewater and its association with sulfonamide antibiotica, which are poorly removed during wastewater treatment. Importantly, *sul1* is commonly embedded in Class 1 integrons, enabling its persistence and transfer between bacterial species even in the absence of selective pressure.

- Sulfonamides are classified as *"highly important antimicrobials"* by the WHO MIA List and fall under the *"access"* category of the WHO EML List, implying medium resistance risk. Although not listed in the WHO 2024 BPPL, sulfonamides remain widely prescribed and are recommended as first- or second-line treatments [9]. Their monitoring is thus crucial, especially as *sul1* is also highly abundant in hospital wastewater and surface waters, pointing to inputs from both clinical use and veterinary/agricultural sectors.The *vanA* gene was the among the top 10 cited ARG targets in wastewater, mainly detected in hospital effluents, agricultural wastewaters, and urban wastewater treatment plants (UWWTPs). *vanA* mediates resistance to glycopeptides (e.g., vancomycin), which are last-resort treatments for severe Gram-positive infections.

- Glycopeptides are ranked among the antimicrobials with the highest risk for AMR development in the WHO MIA List.
- Vancomycin is categorised under "*watch*" antibiotics in the WHO EML List, reflecting its high resistance selection potential.
- The WHO 2024 BPPL highlights the rise of vancomycin-resistant Enterococcus faecium (VRE) as a major concern.

Given this evidence, *vanA* should be prioritised as a core target in wastewater surveillance to inform antimicrobial stewardship.

Tetracycline-resistance genes (*tetA, tetM, tetW*) also appeared prominently, particularly in surface waters receiving wastewater effluents. While tetracyclines are not prioritised in the WHO 2024 BPPL and show lower resistance potential according to the WHO EML and MIA Lists, monitoring remains relevant within a One Health framework, given their broad dissemination across environmental compartments.

The high prevalece of the *ermB* gene highlights the importance of monitoring macrolide resistane. Macrolidesare listed as "critically important antimicrobials" in the WHO MIA List. Their clinical importance is reinforced by the inclusion of macrolide-resistant Streptococcus pneumoniae and macrolide-resistant Group A Streptococci as new entries in the 2024 BPPL.

Other ARGs of particular concern include *mcr-1*, *qnrS* and *mecA* conferring resistance to colistin, quinolones and methicillin-resistant *Staphylococcus aureus* (MRSA), respectively.
- Colistin is categorised as a 'highest priority critically important antimicrobial' (WHO MIA List).
- Quinolones are listed as highly critical in the WHO 2024 BPPL.
- MRSA is one of the most prevalent drug-resistant pathogens, particularly in high-income countries.

The frequent detection of *mecA* in hospital wastewaters is consistent with this burden.

Together, these findings underline the public health importance of prioritising ARG monitoring in wastewater environments, both for early-warning surveillance and for guiding the implementation of AMR mitigation strategies under the UWWTD recast.

Globally, efforts are underway to harmonise AMR monitoring in order to better characterise its spread and to design effective mitigation strategies. However, the literature reviewed indicates that, although many EU Member States acknowledge the importance of standardised methodologies and the collection of associated metadata, actual practices remain largely heterogeAneous and fragmented. Within the EU Member States submissions, the GLASS (WHO Global Antimicrobial Resistance and Use Surveillance System) and One Health frameworks were cited in contributions from Austria, Belgium, and France. An overview of the standardisation efforts reported by individual Member States is provided in Annex 2.

Standardisation was found to occur predominantly at the national level, often through the establishment of national monitoring protocols. For example, in France the "AMR-Env" group was established under the French Priority Research Programme on Antimicrobial Resistance, with the objective of developing harmonised approaches for the collection and analysis of samples from natural environments.

By contrast, explicit references to international surveillance protocols or guidelines developed by standardisation bodies such as CEN, ISO, or ASTM were largely absent. Two notable exceptions were: 1. Finland, which reported active participation in ISO/TC 147/SC 4 ("Microbiological methods") for international method development;

2. France, which indicated compliance with ISO standards for the measurement of micropollutants and ARGs.

Additionally, some Member States (Ireland, Italy) reported the adoption of standardised microbiological guidelines from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Efforts to promote harmonisation of data sharing, methodologies, and nomenclature were also identified. Three Member States reported using specialised databases and software tools to support comparability and interoperability of results. Specifically:

1. Germany reported use of the NORMAN ARB&ARG database;
2. Bulgaria reported applying MetaCompare2.0;
3. Sweden reported use of both the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder.

Descriptors for monitoring ARGs

Urban wastewater is increasingly recognised as a critical conduit for ARGs, providing valuable insights into the prevalence and dissemination of AMR within human and animal populations. To assess and manage AMR risks, Member States have employed a variety of descriptors and monitoring parameters tailored to their national objective. These descriptors are essential for: (i) quantifying the occurrence and dynamics of ARGs in wastewater environments;(ii) facilitating comparability of results across studies and countries; (iii) informing interventions, including intersectoral coordination, regulatory actions (e.g. restrictions on over-the-counter antibiotics), hygiene standards, and public education campaigns.

From the survey and literature review, a set of commonly used descriptors could be identified across Member States (Annex 2): Bacterial counts, expressed as CFU per mL of wastewater;

- Absolute ARG abundance, expressed as gene copies per mL of wastewater;
- Relative ARG abundance, normalised to 16S rRNA gene copies, per ng DNA, or per mL of total DNA;
- *intI1* gene, included as a marker of horizontal gene transfer (HGT) and ARG mobility;
- Metadata descriptors, including sampling date, location, analytical methods used, normalisation procedures, and antimicrobial susceptibility thresholds.

In addition to these common descriptors, five Member States reported using further parameters to capture more specific aspects of AMR in wastewater:

- Austria and Belgium calculated the log reduction of ARG abundance between influent and effluent of wastewater treatment plants (WWTPs) to evaluate treatment efficiency.
- France measured the abundance of antibiotic residues (ng/L) in wastewater, linking ARG presence with selective pressure from antimicrobial compounds.
- Bulgaria employed Chao richness estimators to assess bacterial diversity associated with ARG presence.
- Portugal used fractional quantification, distinguishing ARGs located in bacterial DNA from those carried by phages, thereby assessing ARG transfer potential via bacteriophages.

These extended descriptors provide complementary perspective: from WWTP performance in ARG reduction to population-level antibiotic consumption, community structure shifts, and the potential for ARG transfer across vectors. When combined with the standardised ARG parameters, they contribute to a more holistic and integrated assessment of AMR dynamics in wastewater environments.

4. Recent AMR monitoring initiatives

Efforts to monitor antimicrobial resistance (AMR) in the environment have expanded considerably in recent years, driven by the recognition that AMR is a cross-sectoral challenge requiring standardised surveillance and coordinated action. At the global level, international organisations such as the WHO, FAO, and WOAH have developed frameworks that promote harmonisation of methods, integration of environmental data into One Health monitoring systems, and comparability across countries. These initiatives provide both technical protocols and institutional models that are highly relevant for the implementation of AMR surveillance under the recast Urban Wastewater Treatment Directive (UWWTD). Among them, the WHO's "Tricycle" and EU-WISH Join Action initiative have gained particular traction as pragmatic global entry points for environmental AMR monitoring.

4.1. "WHO Integrated Global Surveillance on ESBL-producing *E. coli* (Tricycle Protocol)

The WHO Global Tricycle Surveillance protocol [13] provides a standardised, trans-sectoral framework for monitoring extended-spectrum beta-lactamase-producing Escherichia coli (ESBL-Ec) as an indicator organism for AMR. Developed jointly by the WHO, the Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (WOAH), it is designed to facilitate comparable, affordable, and scalable surveillance across countries, while anchoring AMR monitoring firmly within the One Health approach.

Surveillance Focus:

The protocol targets ESBL-Ec in three priority domains:

- Humans (clinical and community sources),
- The food chain (animals and food products), and
- The environment (urban and animal wastewater, as well as river water).

These domains were selected to represent the major pathways of AMR transmission.

Methodology: The approach relies primarily on culture-based methods, focusing on the detection and quantification of ESBL-Ec in environmental samples, including urban wastewater. Standardised procedures are provided for sample collection, media preparation, and bacterial isolation. While the protocol does not initially require molecular methods (e.g. PCR, WGS), their integration is recommended in subsequent phases for deeper characterisation.

[13] WHO Global Tricycle Surveillance report: https://iris.who.int/bitstream/handle/10665/340079/9789240021402-eng.pdf?sequence=1.

Implementation: Countries adopting the protocol are encouraged to establish a national coordinating group, typically involving microbiologists, epidemiologists, and national focal points. The WHO provides technical assistance through regional and global offices to support implementation and ensure comparability across sites.

Data Management and Analysis: The protocol promotes the use of *WHONET software* for standardised data management, ensuring consistent collection, storage, and sharing of results. The data are used to assess trends in ESBL-Ec prevalence and to guide targeted AMR interventions.

Integration with Other Systems: The Tricycle protocol is closely linked with the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) and with other UN-supported surveillance frameworks, facilitating interoperability and allowing additional "satellite projects" (e.g. molecular studies, environmental expansion) to build on the core design.

Challenges and Considerations: The report highlights challenges related to the complex ecological dynamics of AMR, noting that causal links between sectors cannot always be inferred. It stresses the need for transparency in data interpretation and cautions against the risk of stigmatising specific countries, populations, or sites.

Relevance to the recast Urban Wastewater Treatment Directive:

The Tricycle protocol offers several points of synergy with the objectives of the recast UWWTD:

- It operationalises the One Health mandate by explicitly integrating environmental AMR monitoring with human and animal health sectors.
- It provides a tested, standardised framework that could inform the development of EU-wide wastewater AMR surveillance methodologies.
- It offers technical and methodological support, as well as an international platform for data comparability and policy coordination at regional and global scales.

4.2. EU-WISH

The EU-WISH[14] is a 36-month Joint Action under the EU4Health programme (November 2023 – October 2026), coordinated by the Statens Serum Institut (SSI, Denmark) with the participation of 62 institutions from 26 countries. The initiative is designed to strengthen the EU's capacity for wastewater-based public health surveillance and to complement national systems by promoting knowledge exchange, best practices, and harmonised approaches. Its scope goes beyond antimicrobial resistance (AMR) to include emerging pathogens, viruses, chemical substances, and other health-related biomarkers. EU-WISH also collaborates with EU-JAMRAI 2 [15], reinforcing the environmental component of AMR surveillance within the One Health framework.

[14] EU-WISH - Integrated wastewater monitoring for public health in the EU - EU-WISH

[15] EU-JAMRAI - The joint actions EU-WISH and EU-JAMRAI 2 initiate mutual collaboration | EU-JAMRAI

Surveillance Focus:

The project targets a broad range of public health threats, with emphasis on AMR, ESBL-producing E. coli, carbapenemase-producing Enterobacteriaceae (CPE), as well as respiratory viruses, polioviruses, non-polio enteroviruses, and selected chemical markers. For AMR, the central goal is to use wastewater as a complementary surveillance system to detect trends in resistance within human populations, thereby enhancing epidemiological intelligence.

Methodology:

The primary analytical methods reported in operative programmes were:

- *Whole genome sequencing (WGS)* of bacterial isolates cultured from wastewater;
- *Quantitative PCR (qPCR*) targeting specific ARGs associated with ESBL-Ec and CPE. These approaches allow for both broad genetic characterisation and targeted detection, respectively.

Implementation:

The project was launched in February 2024 in Athens. A mapping survey conducted the same year revealed that 8 of 27 countries (30%) currently operate wastewater AMR surveillance programmes, ranging from exploratory research projects to institutionalised national initiatives.

The first capacity-building workshop took place in Lisbon in October 2024, focusing on harmonisation of methodologies and training, with participation also extended to low- and middle-income countries.

Data Management and Use:

The primary objective of these surveillance systems is to provide data on AMR occurrence and trends to inform public health strategies and epidemiological assessments. However, the survey highlighted that integration of wastewater-based AMR data into national decision-making frameworks remains limited. The translation of surveillance results into actionable public health responses is still under development. Furthermore, EU-WISH makes use of the following work packages: (i) “Work Package 5” maps existing national wastewater surveillance systems and stakeholders and develops priority target lists tailored by region and surveillance objectives, (ii) “Work Package 7” focuses on technical harmonisation, including standardised protocols for sampling frequency, analytical workflows, and laboratory methods.

Challenges and Considerations:

The EU-WISH survey identified several key barriers:

- Lack of integration of wastewater AMR data into established public health surveillance systems;
- Limited duration of projects and reliance on temporary funding, undermining sustainability;
- Difficulty in operationalisation, particularly in moving from research findings to applied decision-support tools.

Relevance to the UWWTD Recast:

EU-WISH highlights both the potential and the current fragmentation of wastewater-based AMR surveillance in Europe. Its findings emphasise the need for:

- Stable funding and institutionalisation of AMR wastewater monitoring;
- Standardised methodologies across Member States;
- Mechanisms to translate surveillance results into public health action.

5. Collaborative Frameworks in Antimicrobial Resistance Monitoring: The Roles of Eionet and TATFAR

5.1 European Environment Information and Observation Network (Eionet)

Surveillance Focus:

The 2025 Eionet pilot study [16] aimed to establish a harmonised framework for monitoring antimicrobial resistance (AMR) in European surface waters and urban wastewater. Conducted across ten self-funded countries, the study focused on rivers downstream of urban wastewater treatment plants (WWTPs). The primary objective was to develop methodologies for collecting and reporting AMR data, supporting revisions of EU water directives.

Methodology:

The pilot study employed a dual approach combining culture-based techniques and quantitative PCR (qPCR) analyses. A strategically selected panel of six core antimicrobial resistance genes (ARGs) was targeted:

- *16S rRNA*
- *intI1*
- *aadA1*
- *ermB*
- *blaCTX-M1*
- *vanA*

Additionally, three optional genes were included:

- *tetW*
- *sul1*
- *blaKPC*

Sampling focused on rivers downstream of urban WWTPs, and the study applied the *WHO Tricycle protocol*.

Implementation:

Participating countries implemented the harmonised framework at representative WWTP sites and surface water locations. Challenges encountered included funding limitations, varied national

[16] Pilot study on antimicrobial resistance monitoring in European surface waters - Final report of the Eionet Working Group

capacities, and differing technical expertise among Member States. Despite these challenges, the pilot study was viewed as a successful step toward EU-wide AMR monitoring.

Key Findings:

- *Spatial Gradients*: Clear spatial gradients in ARG abundance were observed, reflecting variations in human impact across sites.
- *Gene Removal Efficiency*: Wastewater treatment effectively reduced both E. coli and ESBL-producing E. coli (ESBL-Ec) by over 99%.
- *Methodological Variability:* Significant differences in laboratory practices and technical capabilities were detected, highlighting the need for harmonisation.
- *Data Integration Potential:* The pilot demonstrated the feasibility of combining culture-based and molecular methods to provide a robust, multi-dimensional picture of AMR in wastewater and surface waters.
- *Recommendations:* Establishing standardised protocols and quality assurance/control frameworks is critical for generating reliable, comparable data to support policy and public health action at the EU scale.

Sampling Considerations:

- *Sampling Protocols:* The study employed the WHO Tricycle protocol for culturing due to the absence of standardized EU methods for water.
- *Sampling Locations*: Sampling focused on rivers downstream of urban WWTPs, providing insights into the impact of effluent on surface water quality.
- *Sampling Frequency*: The study involved sampling at multiple sites over a period, though specific details on the frequency and duration of sampling were not provided in the available sources[7].

Data Management and Reporting:

- *Data Reporting Template*: A custom data reporting template aligned with the European Environment Agency's WISE-6 model was developed, facilitating standardized data submission across participating countries .
- *Data Harmonization*: The study aimed to harmonise methodologies for sampling, analysis, and reporting, supporting revisions of EU water directives.
- *Quality Assurance:* The study highlighted the need for rigorous quality assurance and control (QA/QC) measures to ensure data comparability and reliability on a Europe-wide scale.

Cost Considerations:

- *Method Selection:* Quantitative PCR (qPCR) was used as the main method for its cost-effectiveness, enabling the detection of key antimicrobial resistance genes (ARGs) across multiple sites.
- *Funding Challenges*: The pilot study faced challenges including funding limitations, varied national capacities, and differing technical expertise among participating countries.

- *Resource Allocation:* Despite these challenges, the study was viewed as a successful step toward EU-wide AMR monitoring, indicating that with appropriate resource allocation, such initiatives can be effective.

5.2 The Transatlantic Taskforce on Antimicrobial Resistance (TATFAR)

Surveillance Focus:

Established in 2009 and expanded over time to include the EU, U.S., Canada, Norway, and the UK, TATFAR [17] adopts a One Health perspective for AMR surveillance, explicitly acknowledging the importance of environmental vectors—including water systems—as integral to the AMR ecosystem.

Methodology & Harmonisation Efforts:
Although TATFAR does not prescribe standardised monitoring protocols for AMR in water, it actively facilitates harmonisation through knowledge exchange.
The Taskforce:

- Shares methodologies including culture-based detection, qPCR, and genomic approaches.
- Emphasises the development and alignment of QA/QC standards, target gene panels (e.g. ESBL and carbapenemase genes), and normalisation practices in sampling (essential measures for transatlantic data comparability).

Implementation and Collaborative Mechanisms:

TATFAR's structure is designed to foster ongoing coordination:

- It issues progress reports and jointly developed resources, such as those on antibiotic use metrics, stewardship strategies, and surveillance frameworks.
- It hosts periodic meetings and workshops, including a significant virtual conference in September 2021 that convened partners from all member regions (EU, U.S., Canada, Norway, UK) alongside WHO and other stakeholders. The meeting set the stage for its 2021–2026 implementation plan Public Health.

Data Management & Sharing:
While TATFAR doesn't operate its own data platform, it promotes data interoperability across jurisdictions by aligning surveillance priorities, collaborating on surveillance tool development, and reinforcing frameworks for sharing methodologies and outcomes.

Challenges & Strategic Directions:
TATFAR's recent progress reports highlights future priorities, including:

- Advancing wastewater surveillance for environmental AMR.
- Enhancing cross-sector collaboration on stewardship, diagnostics, and AMR transmission routes.

[17] Transatlantic Task Force on Antimicrobial Resistance | HHS.gov

- Addressing methodological gaps in data comparability and expanding surveillance coverage.

Relevance to the recast UWWTD

TATFAR's role offers valuable insights for the recast UWWTD including:

- It demonstrates how inter-jurisdictional collaboration can align surveillance approaches across differing regulatory frameworks.
- The emphasis on fostering harmonised methodologies, QA/QC practices, and target gene panels informs the development of EU-wide standardisation.
- TATFAR's strategic positioning can support **the EU directive's ambition** to integrate wastewater surveillance within One Health frameworks, enabling comparative data and collective response strategies at a transatlantic level.

6. International standardisation

International standardisation for monitoring AMR is in place, with several organisations involved and development underway to improve harmonisation for wastewater and other sectors including water and food, and (13). Currently in place are:

ISO (International Organization for Standardization): ISO develops international standards through technical committees and consensus among member bodies, emphasising harmonised methods based on collective agreement and formal balloting. Regarding antimicrobial resistance (AMR), several standards relevant to healthcare organization management and laboratory quality assurance are currently in draft or committee stages.
Dedicated ISO standards specifically targeting AMR monitoring in wastewater and other water sources are still in the early phases of development. For instance, work is ongoing within relevant technical committees, with draft standards expected to advance through the committee stages over the next 1-3 years. However, precise delivery dates have not yet been published.

In the meantime, existing ISO protocols provide foundational guidance for pathogen detection and antimicrobial susceptibility testing. These include ISO 19458, which covers microbiological sampling of water; ISO 11731, focused on Legionella detection; and ISO 20776-1 and 20776-2, which define antimicrobial susceptibility testing methods originally designed for clinical isolates but widely adapted for environmental bacterial characterisation. These established protocols serve as valuable interim references while dedicated AMR surveillance standards continue to be developed.

CEN (European Committee for Standardization): CEN develops regionally tailored standards that align with European regulatory priorities and environmental health needs, often complementing ISO methods. CEN is actively engaged in developing and updating standards supporting wastewater treatment and water monitoring, including aspects crucial for antimicrobial resistance (AMR) surveillance[18].

Key standards include EN ISO 19458 (Water quality: Sampling for microbiological analysis), EN ISO 9308-1/2 (Coliform enumeration), EN 12566, ISO 20776 (Parts 1 and 2), and various standards under the technical committee CEN/TC 230. Together, these provide a comprehensive framework for chemical and microbiological water quality monitoring, forming a strong basis for AMR surveillance initiatives [19].

Currently, several AMR-related CEN standards are under development or review, aligned with broader European strategies to mitigate AMR risks in the environment. Although precise delivery dates are not yet publicly available, ongoing work supports enhanced environmental surveillance capacities, especially focusing on wastewater treatment plants and surface waters [20].

This progress is closely coordinated with European One Health AMR action plans and associated research partnerships promoting standardised surveillance methods. In particular, the European Joint Programming Initiative on AMR (JPIAMR) has advanced harmonised molecular and culture-based

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[18] https://pmc.ncbi.nlm.nih.gov/articles/PMC9261269/

[19] Update on AMR-related issues in several environmental policy contexts

[20] Call-to-Action-of-the-European-Network-on-Infection-Prevention-and-Antimicrobial-Resistance-ENIPAR.pdf

surveillance techniques relevant to wastewater-based AMR monitoring. Key outputs informing this work include the development of transnational research agendas, inter-laboratory studies, and methodological guidelines. Specific projects include: (i) RESERVOIR, (ii) BALTIC-AMR, (iii) DECODE, and (iv) OASIS. Together, these initiatives support the harmonisation efforts presented in this report [21,22,]

ASTM International: ASTM International develops standards for a wide range of industries including environmental testing. Certain ASTM standards, such as ASTM D5465, are commonly used in conjunction with ISO and CEN methods for detecting indicators like *coliforms* and *E. coli* in water. However, specific ASTM methods explicitly focused on antimicrobial resistance (AMR) detection in water are still under development and delivery timelines remain to be announced.[23] These developments aim to complement existing international standards and address gaps in environmental AMR surveillance.[24]

National and regional for antimicrobial resistance (AMR) monitoring in water are used variably across countries. For example, in Germany, the DIN 38411 standard series—including methods for microbiological examination of water, wastewater, and sludge—is commonly applied for bacterial indicator analysis and AMR-related monitoring [25]. These methods include both culture-based techniques and modern identification approaches, ensuring comprehensive surveillance of microbial communities and resistance traits.

In the United States, AMR monitoring in water typically employs a combination of culture-based assays, quantitative PCR (qPCR), and metagenomic sequencing. National regulatory and public health agencies, such as the Environmental Protection Agency (EPA) [26] and the Centers for Disease Control and Prevention (CDC)[27], provide various protocols which guide these assessments. However, these protocols are diverse and sometimes lack full harmonisation for inter-laboratory comparability. Ongoing national efforts in the US focus on developing standardised frameworks to ensure consistent and comparable AMR surveillance data across institutions. These frameworks integrate molecular and culture-based methods to address current gaps and improve environmental AMR monitoring comprehensiveness. While precise timelines for the completion and delivery of these frameworks are not yet publicly disclosed, stakeholder collaborations suggest active development stages with expected advancements in the near future. The Joint Research Centre (JRC) involvement with the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) aims to facilitate more detailed information exchange and alignment of standards between Europe and the US, potentially enhancing cross-border cooperation and technical harmonisation.

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[21] How to establish a hospital wastewater surveillance program for antimicrobial resistance: Current experience and future knowledge gaps

[22] Measuring water pollution effects on antimicrobial resistance through explainable artificial intelligence - ScienceDirect

[23] A one health approach for monitoring antimicrobial resistance: developing a national freshwater pilot effort - PMC

[24] A one health approach for monitoring antimicrobial resistance: developing a national freshwater pilot effort - PMC

[25] DIN Standards Series - Results

[26] linking_epa_to_a_one_health_focused_national_scale_monitoring_of_antimicrobial_resistance.pdf

[27] Strengthening Surveillance - Combating Antimicrobial Resistance and Protecting the Miracle of Modern Medicine - NCBI Bookshelf

7. National Obligations for AMR Monitoring in Aquatic Systems: EU-27 Comparative Analysis

There is currently no explicit EU regulatory requirement for monitoring or controlling antibiotic residues or antimicrobial resistance genes (ARGs) in aquatic environments. However, this gap is acknowledged, and the topic is under active discussion, with ongoing efforts to develop recommendations for action[28]. The EU Water Framework Directive (WFD) [29] provides a mechanism for setting Environmental Quality Standards (EQS) for water pollutants, but AMR selection risk and antibiotic residues are not currently included among binding EQS. The WFD does allow Member States to designate additional river basin-specific pollutants and establish corresponding national EQS adapted to local conditions. Ongoing review processes aim to assess the need for including AMR-related indicators and antibiotic residues within future EQS frameworks.
The analysis of national obligations is based on a structured review of Member States' legislation, policy instruments, and institutional mandates relevant to AMR monitoring in aquatic environment, including in wastewater. This work has identified whether binding national obligations exist, and highlights relevant national plans and instruments, EU linkages, and scientific references. Data were collected through desk research of national legal databases and government portals, scientific literature analysis, review of national-level directives, OECD and ECDC reports, consultation of the WHO TrACSS Global Database[30] and results from the 2024 WHO Tracking Antimicrobial Resistance Country Self-Assessment Survey[31]. Table A2 in Annex 3 collects these findings. The dataset may not reflect all ongoing initiatives due to variability in national reporting and evolving legislation.

Key results include:
(i) With regard to urban wastewater, no EU Member State has yet established binding national regulations that explicitly mandate routine AMR monitoring at wastewater treatment plants; (ii) Sweden is currently the only EU Member State with a binding legal requirement for AMR-related monitoring in surface waters, implemented through Environmental Quality Standards (EQS). For example, ciprofloxacin is regulated with a Maximum Allowable Concentration EQS (MAC-EQS) of 0.1 µg/L. (iii) All other Member States currently rely on voluntary, research-driven, or pilot-based initiatives for urban wastewater AMR monitoring, but also include aspects of surface water surveillance and broader water contamination control under the One Health approach. (iv) EU-level coordination is reflected in Member State participation in initiatives such as JPIAMR [32], EU-JAMRAI [33], EARS-Net [34], and alignment with the EU AMR Action Plan and WHO Global Action Plan on AMR; (v) the European Commission and environmental agencies recognise the health risk posed by environmental AMR and recommend establishing methods and standards for risk-based regulation of antimicrobial residues in water.

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[28] European Environment Agency (EEA) report Water signals — European Environment Agency

[29] Water Framework Directive - Water Framework Directive - European Commission

[30] Global Database for Tracking Antimicrobial Resistance (AMR) Country Self- Assessment Survey (TrACSS)

[31] Results from the 2024 Tracking Antimicrobial Resistance Country Self-Assessment Survey (TrACSS)

[32] EU AMR projects JPIAMR, OHAMR

[33] EU AMR project EU-JAMRAI

[34] European Antimicrobial Resince Surveillance Network (EARS-Net)

8. Analytical methods for monitoring AMR in water: strengths and limitations

In the absence of harmonised international standards, a diverse suite of analytical methodologies is currently employed for antimicrobial resistance (AMR) monitoring in water environments. These methodologies span traditional culture-based assays to cutting-edge molecular and genomic approaches, each characterised by unique advantages and constraints regarding sensitivity, breadth of detection, interpretability, and operational feasibility for regulatory or research use.

Culture-based methods

Culture-based methods remain foundational for detecting viable antimicrobial-resistant bacteria (ARB) and generating quantitative phenotypic resistance profiles. Standardised protocols such as coliform enumeration (e.g., EN ISO 9308) and the World Health Organization's (WHO) Tricycle protocol (22), endorsed by the European Environment Information and Observation Network (Eionet), facilitate targeted detection of priority bacterial species and resistant phenotypes (e.g., ESBL-producing *E. coli*). These approaches afford direct evidence of viable, culturable resistance reservoirs and allow antimicrobial susceptibility testing critical for clinical and epidemiological contexts. Nonetheless, they are limited by lengthy incubation requirements, labour intensity, and an inability to capture viable but non-culturable (VBNC) bacteria and fastidious or slow-growing taxa prevalent in complex aquatic microbiomes, potentially underestimating resistance diversity and abundance in environmental matrices.

Molecular approaches

Molecular methods, especially <u>quantitative polymerase chain reaction</u> (qPCR), have rapidly gained prominence due to their high sensitivity, specificity, and rapid turnaround in detecting known antibiotic resistance genes (ARGs). qPCR facilitates high-throughput monitoring across large sample sets, independent of bacterial viability, making it suitable for broad environmental surveillance. European pilot studies (like Eionet) have demonstrated feasibility for harmonised qPCR protocols incorporating standardised primer-probe sets, thermocycling conditions, and synthetic calibration standards (e.g., gBlocks), which improve inter-laboratory comparability and data robustness. However, qPCR's reliance on prior knowledge of target ARG sequences restricts detection of emerging or novel resistance mechanisms, and molecular detection does not inherently confirm phenotypic resistance expression. The development of certified reference materials, such as those currently underway at the JRC, will further enhance quality assurance and method standardisation.

<u>High-resolution genomic techniques,</u> comprising whole genome sequencing (WGS) and shotgun metagenomics, offer transformative potential for characterising the entire resistome, mobile genetic elements, and microbial community structure in water environments. These comprehensive approaches enable discovery of novel ARGs, facilitate strain-level source tracking, elucidate gene mobility, and support refined risk assessment models within integrated One Health frameworks. Recent reviews and ongoing monitoring programs emphasise WGS and metagenomics as critical to unravelling AMR dynamics across environmental compartments. Nevertheless, their widespread adoption is hindered by high financial and computational costs, the necessity for specialised bioinformatics expertise, extended data processing times, and the current absence of internationally accepted standardised analytical pipelines and quality control measures.

Emerging or complementary technologies: Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry, while widely used in clinical microbiology for microbial identification and resistance profiling, remains underexplored in environmental contexts due to instrumentation costs and the need for protocols tailored to water matrices. Lab-on-a-chip and microfluidic platforms represent innovative tools for on-site AMR detection by integrating molecular assays into portable, miniaturised systems. These technologies could enable near real-time monitoring at wastewater treatment plants or environmental hotspots, although most remain in early developmental stages and require thorough validation for consistent performance across diverse environmental conditions. Similarly, pilot systems such as smart-chip Resistomap platforms offer potential for multiplexed resistance profiling, though they also require further technical refinement before they can be implemented widely.

Overall, the current landscape of AMR monitoring in wastewater benefits from a complementary toolbox integrating culture-based methods, targeted molecular assays, and broad genomic approaches. Future efforts should prioritise international harmonisation of protocols, validation and certification of reference materials, and the strategic integration of emerging technologies. Such coordinated strategies are essential to enhance sensitivity, specificity, and scalability of environmental AMR surveillance. Additionally, incorporating comprehensive One Health perspectives entails expanding ARG monitoring to encompass resistance determinants beyond traditional antibiotic targets, including those linked to antifungals, antivirals, and antiparasitic agents, as well as underexplored microbial groups such as fungi, viruses, and protozoa.

9. Discussion

In response to the requirements of the Article 17(3) of the recast UWWTD the JRC has undertaken a comprehensive scientific effort to support the development of a harmonised methodology and certified reference material for monitoring antimicrobial resistance (AMR) in urban wastewater, providing a crucial contribution to the effective implementation of the Directive. Anchored in the One Health framework, this work directly contributes to the European Union's broader objectives on public health surveillance and environmental protection.

This report aims to provide the technical foundation for consistent and comparable AMR surveillance across EU Member States, ahead of the 2030 reporting obligations. Through scientific literature analysis, active stakeholder consultation, and providing technical support for the implementing act, the JRC has delivered key recommendations to facilitate the implementation of AMR monitoring in urban wastewater, laying the groundwork for effective regulatory action and long-term health risk mitigation.

Although AMR surveillance has traditionally focused on human and animal health, increasing evidence highlights the natural environment as a critical reservoir and potential driver of AMR emergence and dissemination [35]. Consistent with the One Health approach, systematic environmental monitoring, particularly of antimicrobial residues and resistance determinants in urban wastewater, is indispensable to elucidate the role of environmental contamination in AMR proliferation, promote prudent antimicrobial use, inform effective infection prevention strategies and assess associated risks to human health.

To effectively confront this challenge, it is essential to identify critical control points, establish reliable surveillance frameworks, and deploy technological solutions aimed at preventing environmental contamination by antibiotic-resistant bacteria and bacterial ARGs.
In this regard, monitoring both the influent and effluent of WWTPs can provide critical insight into AMR inputs from the community and, the effectiveness of treatment processes in reducing resistance loads before environmental discharge.

Analysis of scientific literature and Member States' initiatives reveals substantial heterogeneity in AMR monitoring methodologies within urban wastewater contexts. Variations exist in sampling strategies (composite vs. grab samples), normalisation procedures (accounting for flow, volume, or temporal factors), and monitored matrices, reflecting divergent national contexts and objectives. Wastewater influent and effluent from treatment plants consistently emerge as primary matrices due to their critical role in reflecting AMR reservoirs and treatment efficacy.

Culture-based methods remain valuable for isolating viable bacteria and conducting population-level analyses. However, their limitations in standardisation, scalability, and time-efficiency restrict their broader application. In contrast, qPCR offers a faster turnaround, greater reproducibility, and targeted approach for quantifying defined ARGs. It is particularly well-suited to wastewater environments,

[35] https://www.frontiersin.org/articles/10.3389/fmicb.2021.766242/full

where bacteria may be viable but non-culturable, and where rapid detection of resistance markers is essential.

Findings from the Eionet pilot study also support the feasibility of deploying standardised qPCR methods in a harmonised European monitoring framework. This includes consensus development around primer-probe sets, thermocycling protocols, and the use of synthetic standards (e.g., gBlocks) for calibration and inter-laboratory comparability. These efforts can be further strengthened through certified reference materials, e.g., the one currently being developed by the JRC, which will strengthen quality assurance and intercalibration efforts.

Across Europe, Member States reported a total of 189 ARG targets identified in environmental matrices, emphasising the complexity and scale of AMR in wastewater systems. Drawing on this extensive dataset and applying a prioritisation framework that considers both public health relevance (WHO 2024 BPPL) and environmental significance (as identified by the Eionet AMR Working Group pilot study on AMR monitoring in surface waters), the JRC has selected 24 ARG targets for qPCR-based AMR monitoring in urban wastewater plus 1 additional genetic target (*intl1*) in urban wastewater. From this selection, a further selection resulted in 12 key ARGs (Table 3): *blaCTX-M, blaOXA, mcr-1, blaKPC, vanA, ermB, mecA, qnrS, aadA1, sul1, tetA, and dfrA.* The selection reflects a representation of one to three ARGs per major antibiotic class, including beta-lactams (*blaCTX-M, blaOXA, blaKPC*), colistin (*mcr-1*), glycopeptides (*vanA*), macrolides (*ermB*), quinolones (*qnrS*), streptomycin (*aadA1*), MRSA (*mecA*) tetracyclines (tetA), sulfamethoxazole (*sul1*) and trimethoprim (*dfrA*).

Among these, particular attention is now being directed towards four high-priority ARGs*: blaCTX-M, sul1, blaKPC,* and *ermB*, which are considered especially relevant under the One Health framework, as they are also recognised as priority targets by both the WHO 2024 BPPL and the Eionet AMR Working Group (Table 2).

Table 2. Priority ARG targets proposed for environmental monitoring under the One Health framework. This selection reflects ARGs of high relevance to both public health and environmental dimensions, based on alignment with WHO priority pathogens and Eionet AMR Working Group recommendations. The table highlights four key targets: *blaCTX-M*, *blaKPC*, *sul1*, and *ermB*, for harmonised qPCR-based surveillance in urban wastewater systems across Europe.

Relevance	#	ARG target	Antibiotic	# of citations	WHO 2024 BPPL	Eionet AMR working group
One Health	1	*blaCTX-M*	beta-lactams	55	Yes	Yes
	2	*sul1*	sulfamethoxazole	35	Yes	Yes
	3	*blaKPC*	beta-lactams	30	Yes	Yes
	4	*ermB*	macrolides	22	Yes	Yes

Source: EC-JRC

Building on these insights, this report recommends the implementation of a qPCR-based AMR surveillance strategy. The JRC is currently working with its partners to validate a suitable qPCR-method on the reported targets.

Ensuring data quality and comparability, clear guidance on analytical validation (e.g., qPCR efficiency thresholds) is necessary, alongside interlaboratory proficiency testing and the use of reference materials. The routine reporting of qPCR results, including Ct values or gene copies per millilitre (gene/mL), should adhere to simplified, standardised formats similar to those used for traditional chemical concentration reporting.

Equally important is the facilitation of data sharing. International platforms such as the WHO GLASS and the EU's Super-Site Sentinel System operated by JRC, HERA and HaDEA, are pivotal in facilitating data exchange, collaborative monitoring, and dissemination of best practices across countries. Moreover, capacity-building initiatives are critical to strengthen expertise, particularly in countries with less experience in environmental and wastewater AMR monitoring.

Furthermore, metagenomics has strategic potential in environmental AMR monitoring and warrants targeted pilot studies and research investments. At present high costs, technical complexity, and data-intensive requirements are currently limiting the widespread application of metagenomics. Voluntary exploration of metagenomic approaches, alongside other emerging technologies such as lab-on-a-chip platforms, within national surveillance programmes is strongly encouraged where resources and technical capacity permit. These efforts are further supported by initiatives such as the JRC's offer of technical assistance at no additional cost as part of the newly established EU Sentinel System, facilitating broader capacity-building and innovation uptake. This includes methodological guidance, access to reference materials, databases, and training opportunities. Such support facilitates broader innovation uptake, accelerates harmonisation of approaches, and reduces the risk that resource-limited countries are excluded from next-generation surveillance developments.

Such exploratory work can inform the refinement of future monitoring frameworks, help uncover novel resistance pathways and broaden the scope of surveillance to include underrepresented microbial domains such as fungi, viruses, and protozoa, thus contributing to a more comprehensive and integrated One Health approach. Sustained and coordinated engagement among EU Member States remains the *conditio sine qua non* for the effective and harmonised advancement of environmental AMR surveillance.

9.1. Preliminary method proposal for AMR monitoring in urban wastewater

Building on the evidence acquired through the reviewed literature, this report proposes a targeted, cultivation-free qPCR monitoring approach for AMR monitoring in wastewater. qPCR offers precise quantification of known ARGs of concern and has already been undertaken in AMR monitoring in large part of EU Member States. This preparatory support reduces entry barriers and facilitates a smoother uptake of harmonised monitoring practices once the implementing acts under the Urban Wastewater Treatment Directive enter into force.

A list of 25 targets has been curated based on the submitted Member States' literature, in conjunction with expert opinion, internal discussions and existing international lists (Table 3).

Table 3. Priority antimicrobial resistance gene (ARG) targets proposed for harmonized AMR monitoring within the One Health framework. ARGs are grouped into four relevance categories—One Health, Environmental, Public Health, and Scientific—based on their alignment with the WHO 2024 Bacterial Priority Pathogens List (BPPL), the Eionet AMR Working Group pilot study on European surface waters, and their citation frequency in the literature. This categorization highlights ARGs of cross-sectoral importance for consistent qPCR-based surveillance in urban wastewater and related environmental compartments. [1] *intI1* is not considered an ARG but is often included due to its association with the spread of antibiotic resistance genes. It is used also as an indicator for horizontal gene transfer (HGT) and transmission of ARGs.

Relevance	#	ARG target	Antibiotic	# of citations	WHO 2024 BPPL	Eionet AMR working group
One Health	1	*blaCTX-M*	beta-lactams	55	Yes	Yes
	2	*sul1*	sulfamethoxazole	35	Yes	Yes
	3	*blaKPC*	beta-lactams	30	Yes	Yes
	4	*ermB*	macrolides	22	Yes	Yes
Environmental	5	*vanA*	glycopeptides	25	No	Yes
	6	*intI1*[1]	-	16	No	Yes
	7	*tetW*	tetracycline	11	No	Yes
	8	*aadA1*	streptomycin	10	No	Yes
Public Health	9	*qnrS*	quinolones	13	Yes	No
	10	*mecA*	MRSA	12	Yes	No
	11	*blaOXA*	beta-lactams	51	Yes	No
	12	*blaNDM*	beta-lactams	37	Yes	No
	13	*blaTEM*	beta-lactams	29	Yes	No
	14	*blaVIM*	beta-lactams	26	Yes	No
	15	*blaIMP*	beta-lactams	15	Yes	No
	16	*blaSHV*	beta-lactams	15	Yes	No
	17	*blaCMY*	beta-lactams	14	Yes	No
	18	*aac(6')-Ib*	quinolones	10	Yes	No
	19	*ermF*	macrolides	10	Yes	No
Scientific	20	*tetA*	tetracycline	25	No	No
	21	*mcr-1*	colistin	23	No	No
	22	*tetM*	tetracycline	20	No	No
	23	*blaGES*	beta-lactams	12	No	No
	24	*dfrA*	trimethoprim	11	No	No
	25	*sul2*	sulfamethoxazole	15	No	No

Source: EC-JRC

The first four targets listed in Table 3 are considered of highest priority and represents the minimum for AMR monitoring in wastewaters

Targets were selected as a result of their One Health impact, highlighted both by the WHO 2024 BPPL and the Eionet AMR working group report on AMR monitoring in European surface waters (also see Table 2 above). As the environment can act both a reservoir and transmission pathway for AMR, environmentally relevant ARGs were lifted as the second group of priority targets; these targets align with the Eionet working group study. ARG targets of public health importance were selected in light of the WHO 2024 BPPL. Finally, to complete the list, five scientifically relevant targets, identified through our revision process and internal discussions, were included. The list includes also *intI1*, which is not considered an ARG, but is an important environmental marker associated with AMR.

Frequency of monitoring

Based on scientific evidence and expert consensus, we recommend monthly qPCR monitoring for both influent and effluent at each urban wastewater treatment plant (UWWTP), resulting in 24 samples per year. This frequency is considered sufficient to capture temporal trends in ARG abundance while remaining feasible in terms of cost and operational resources.

Scientific rationale:

- Temporal resolution: Monthly sampling captures seasonal variations, short-term fluctuations, and potential episodic spikes in ARGs, which are critical for detecting trends, emerging resistance, or public health risks.
- Statistical robustness: Regular sampling provides enough data points to perform meaningful trend analyses and correlation with environmental and epidemiological variables, reducing the risk of misleading conclusions due to outliers.

The proposed cultivation-free qPCR monitoring has been reviewed by experts and is recommended for its ability to rapidly, sensitively, and reproducibly detect ARGs directly from wastewater DNA. This approach supports routine surveillance while avoiding the higher labor and time demands of culture-based methods, effectively balancing data quality, resource allocation, and temporal variability. Here, data quality refers to the reliability, representativeness, and reproducibility of measurements, ensured through standardized sampling, consistent processing, and validated qPCR assays.

More frequent monitoring can provide a detailed understanding of antimicrobial use levels and evolution of AMR genes, but it also requires more resources. Furthermore, the frequency of wastewater monitoring can vary depending on regulatory requirements, the specific goals of the monitoring programme, and the logistical and financial resources available. In this light, frequency of routine monitoring of selected AMR targets may require adjustments in the case of specific events (e.g., seasonal changes, industrial discharges, or public health emergencies), study-related assessments or introduction of regulatory frameworks which dictate specific requirements for AMR monitoring. Therefore, monitoring activities initiated by Member States should be adapted to align with minimum requirements, their scientific objectives and practical considerations.

Metagenomic sequencing

Within the proposed AMR monitoring strategy, metagenomic sequencing will function as a complementary tool, carried out by Member States on a voluntary basis. This allows for comprehensive and unbiased resistome profiling, enabling the detection of both known and emerging ARGs within microbial communities and the adaptation of qPCR monitoring targets to the evolving antimicrobial resistome. As a result, it enhances our understanding on the current environmental AMR profile and evolving resistance mechanisms. Traditional molecular techniques, employed by most European countries, such as qPCR and culture-based approaches, are unable to provide these insights.

Metagenomics offers:

Comprehensive Resistome Profiling: Metagenomics offers the ability to reconstruct a wide array of novel ARGs, offering an accurate and holistic picture of the resistome, that is more comprehensive than other approaches (10). This capability is crucial for identifying emerging threats that currently could go undetected by targeted methods.

Agnostic Methodology: It allows for an unbiased approach which extends our knowledge of ARGs beyond the currently known ones. In the future, reconstruction of ARGs can happen directly from metagenomic data even with a low sequence similarity to known ARGs. Currently, this is still under research, as it requires extensive bioinformatics capability.

Contextual Information: Long metagenomic read can provide some contextual data, such as on plasmids, integrons and co-occurrence of ARGs, which is essential for risk assessment and understanding the broader ecological impact of AMR.

Adaptability and Scalability: As bioinformatics and sequencing technologies advance, metagenomics remains adaptable, ensuring its relevance and scalability for future surveillance needs. The developments in AI and automated bioinformatics of recent years, will help to process and analyse larger quantities of complex data.

There are also some limitations as compared to other techniques. Unless using alternative metagenomic sequencing approaches (e.g. Hi-C metagenomics), 2^{nd} generation short reads are known to be limited in linking resistance genes to their bacterial host. Furthermore, there are frequently repetitive regions surrounding resistance genes making it difficult to identify its mobile genetic element, such as plasmids. 3^{rd} generation sequencing technologies, at least in part, may overcome these difficulties. Finally, metagenomic sequencing is resource-intensive, both in terms of financial cost and data processing requirements (Annex 5).

Monitoring is recommended on a monthly basis for both influent and effluent samples from each WWTP, resulting in a total of 24 samples per year. Biannual monitoring strikes a balance between collecting enough data to build a robust knowledge base, to manage the practical constraints of extensive sequencing and to allow informed decision-making. Furthermore, conducting biannual metagenomic analyses can help capture of potential seasonal variations on the distribution and evolution of the antimicrobial resistance. This frequency enables to identify patterns linked to seasonal factors such as fluctuations in temperature, rainfall, and human activity.

Financial and technical help will be provided to Member States wanting to undertake metagenomics as part of the newly established EU Sentinel System. This includes access to standardised protocols, expert guidance, and personnel training. Such assistance will also help Member States build long-term technical capacity and integrate metagenomic data into routine monitoring alongside qPCR methodology.

9.2 Cost considerations

The cost figures presented here are simplified estimates for assay analysis only; a detailed breakdown of all associated costs is provided in Annex 5.

For the proposed harmonised monitoring of urban wastewater, monthly sampling of both influent and effluent at each wastewater treatment plant (WWTP) is recommended, resulting in 24 samples per year per plant. This sampling frequency balances temporal resolution, resource allocation, and surveillance quality.

Annual Cost Estimates:

Based on published data for standard qPCR assays targeting antimicrobial resistance genes (ARGs), the analytical cost per assay is estimated to range between approximately €100 and €300.[36] Applying this range to the proposed sampling strategy, the annual analytical cost per WWTP would be:

24 samples/year × €100–€300 ≈ €2,400–€7,200 per WWTP per year

These figures reflect assay costs only and assume single-gene or small multiplex panels, as recommended for the harmonised EU approach.

They do not include other essential activities such as (i) sample collection and handling at the WWTP, (ii) transportation to the laboratory, (iii) personnel costs, (iv) reporting and data management, or (v) laboratory accreditation and quality assurance measures. A more comprehensive breakdown of both capital expenditures (CapEx) and operational expenditures (OpEx), including sampling, transport, and additional laboratory processing costs, is provided in Annex 5.

Method Selection and Rationale

- Cultivation-free qPCR is proposed as the standard method because it enables rapid, sensitive, and reproducible detection of ARGs directly from wastewater DNA, without the labour, time, and variability associated with culture-based methods.
- Alternative methods, such as metagenomics, provide broader resistome profiling but remain more expensive (€500–€1,500 per sample) and require specialised bioinformatics support [37]. These can be adopted voluntarily by Member States.

Other methods (e.g., ddPCR, cultivation-based PCR, lab-on-a-chip) are informative but most expensive or experimental and, are not recommended for a baseline EU surveillance programme [38].Overall, qPCR remains the most cost-effective for rapid and targeted analyses, while ddPCR is favoured for precise quantification, particularly for low-copy-number targets. Cultivation-based methods are useful when viability and strain differentiation are necessary, but at the cost of increased time and resources. Institutions with high-throughput needs or involved in detailed microbiome or resistome profiling may have the capacity for combinations of these methods to optimise cost and data quality.

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[36] Current and Future Technologies for the Detection of Antibiotic-Resistant Bacteria - PMC

[37] CARPDM: cost-effective antibiotic resistome profiling of metagenomic samples using targeted enrichment - PubMed

[38] Monitoring-AMR-in-the-EU.pdf

For a more detailed discussion and overview on the estimation and evaluation of costs for different analytical methods for monitoring AMR in wastewater, please refer to the Annex 5.

9.3 Additional considerations

Handling of wastewater samples

Sampling kits are available to filter wastewater samples, followed by either DNA extraction on-site or the cold shipment of samples to specified laboratories. By trapping bacteria in filters under nutrient-free, dry, and cold or frozen conditions, the likelihood of significant changes in the bacterial community is minimised, better reflecting the conditions at the time of sample collection. In general, it is a more accessible and feasible method for sample collection. Centrifugation and subsequent concentration of samples may be another viable option.

Freezing of wastewater samples is a common practice for storage reasons and possible retrospective analyses. However, the thawing process may compromise the integrity of the samples. For example, cell lysis during thawing can lead to an inaccurate representation of the bacterial state compared to the time of sampling. Therefore, this approach is not recommended

9.4 The value of other technologies in AMR monitoring

High-throughput quantitative PCR (HT-qPCR)

HT-qPCR is an qPCR approach which has an increased speed, sensitivity and specificity, and throughput compared to traditional qPCR methods. It allows for the analysis of a large number of samples or targets simultaneously. It excels in detecting and quantifying specific resistance genes, even at low abundance, 10^{-4} to 10^{-5} in proportion to the 16S rRNA genes[39]. HT-qPCR provides standardised quantitative measurements, enabling consistent data collection and quick results, which are essential for timely public health interventions, such as identifying hotspots and trends, issuing clinical alerts, or taking other actions to reduce the risk of AMR spread [40].
In addition, it supports the evaluation of the performance of WWTPs in removing AMR genes before discharge, helping to minimise their release into the water environment. Yet, it is not to be forgotten that HT-qPCR also has its limits, notably the possibility for false positive signals due to the lack of probes, which can be problematic if used for public health and environmental signals [41].

Another example of HT-qPCR technology relevant to public health is the SmartChip qPCR system, which efficiently analyses a wide range of samples and genes, from 12 up to 384 genes simultaneously[42]. However, a higher number of targets results in a great increase in cost per sample. SmartChip qPCR provides standardised quantitative measurements, supported by benchmarking indexes for AMR load in European wastewater samples. SmartChip qPCR provides standardised quantitative measurements, supported by benchmarking indexes for AMR load in European wastewater samples.

[39] Contributions and Challenges of High Throughput qPCR for Determining Antimicrobial Resistance in the Environment: A Critical Review - PMC

[40] Rapid Methods for Antimicrobial Resistance Diagnostics - PMC

[41] High-throughput qPCR profiling of antimicrobial resistance genes and bacterial loads in wastewater and receiving environments - PubMed

[42] SmartScreen-AIS: A high-throughput qPCR chip for nationwide surveillance of aquatic invasive species

Culture-based methods

Culture-based approaches, which involve growing and testing bacterial isolates, continue to play a role in antimicrobial resistance surveillance, particularly for confirming phenotypic resistance. Culturing methods, which can offer as important complementary information and can act as an extension to the proposed monitoring strategy. Firstly, high throughput methods cannot address treatment effectiveness as, at the DNA level, they measure genes from both live and dead bacteria. These can be differentiated on the RNA level. There are modified culture methods emerging on the market which permit measurement of trends over time, while simultaneously measuring treatment effectiveness by accounting only for live bacteria and measuring exposure risk due to live AMR bacteria still in water post-treatment. These functions that PCR cannot fulfill can inform strategies to interrupt environmental transmission pathways and mitigate illness and mortality. There are modified culture methods emerging on the market which permit measurement of trends over time, while simultaneously measuring treatment effectiveness by accounting only for live bacteria and measuring exposure risk due to live AMR bacteria still in water post-treatment. These functions that PCR cannot fulfill can inform strategies to interrupt environmental transmission pathways and mitigate illness and mortality.

In addition, modified culture methods can re-purpose existing surveillance and regulatory infrastructure. In fact, ISO methods are already in use in Europe and around the world. Laboratories with these methods in place can easily accommodate changes with minimal training and investment. This is especially pertinent to low and middle-income countries, where laboratory equipment and skilled personnel for plating methods can have limited and long sample transportation times make it difficult to meet the sample processing requirement (<8 hours from sampling).

Harmonising metagenomic sequencing

Metagenomics provides a comprehensive, unbiased approach to assess complete AMR profiles. However, results can vary depending on the specific analysis workflow used, including the choice of computational tools, databases, and data processing steps.

Sequencing technology: Technologies from different providers such as Illumina, Nanopore, or PacBio can deliver method-specific results.

Data processing: This includes factors such as the depth of sequencing, the bioinformatic pipeline, and the AMR database used to process the data.

Data storage and IT infrastructure: Hosting large quantities of metagenomic data can be expensive for single institutions. Leveraging existing infrastructure, such as European Centre for Disease Prevention and Control’s (ECDC) EpiPulse or developing tools like DG Health Emergency Preparedness and Response Authority’s (HERA) Advanced Technology for Health Intelligence and Action IT System (ATHINA) Platform, could mitigate costs, support data sharing and collaboration. Both ECDC and HERA expressed their interest in receiving such data, but further agreements are needed. The European Nucleotide Archive (ENA) is another feasible option.

Sharing of metadata: While raw sequencing data can generally be made open access without major constraints, the associated metadata linked to urban wastewater systems may raise sensitivities. The metadata referenced here includes not only standard technical information reported under the Urban Waste Water Treatment Directive (e.g., treatment capacity, influent and effluent characteristics), which is often publicly available, but also more detailed or sensitive contextual data. This can encompass socio-economic information, specific infrastructure details, or operational parameters that wastewater operators may be reluctant to share openly due to concerns around infrastructure security or commercial confidentiality. Addressing these concerns and facilitating the participation of such actors is essential, especially when data linked to regulatory or policy frameworks, such as the European Water Acquis, must be accessible to the public.

Rise of artificial intelligence (AI)

Complementing detection practices, machine-learning models can be trained on available datasets to predict the presence and abundance of specific ARGs in wastewater from defined regions. This approach can help prioritise sampling, flag anomalous results for review, and provide early warnings of emerging hotspots. However, the extent to which such models can be applied depends on the availability and quality of data generated under rUWWTD monitoring requirements.

Developments in quality assurance control (AQC)

The review findings underscore that the imperative for standardisation and robust analytical quality control (AQC) tools in monitoring antimicrobial resistance (AMR) in wastewater has never been greater. The adoption of standardised methodologies is critical to ensure consistency and comparability of data across diverse studies and Member States, forming the foundation for effective public health strategies and policies aimed at mitigating the spread of AMR. Addressing this urgent requirement, the Joint Research Centre (JRC) has initiated efforts to expand its catalogue of reference materials tailored for wastewater surveillance. In partnership with the Institut Pasteur, the JRC is developing certified reference materials (CRMs) designed to support both PCR-based methods and metagenomic approaches. A key feature of this development is the integration of an engineered plasmids with well-characterised ARG sequences. These CRMs function as a validated standard enabling laboratories to calibrate their assays and ensure the analytical accuracy and inter-laboratory reproducibility of ARG detection in wastewater monitoring. The adoption of such reference materials is anticipated to harmonise molecular analyses across Member States, fostering greater comparability of AMR data. This initiative significantly strengthens the JRC's capacity to provide standardised tools, thereby facilitating more accurate, reliable assessments of AMR in wastewater and supporting harmonised surveillance outcomes at both the European and global levels.

10.Next steps

The next steps towards supporting the development of the implementing acts for the recast UWWTD include:.

Experimental evaluation:
Although several methods for AMR monitoring are currently in use, this experimental phase is necessary to systematically assess the proposed harmonized methodology's performance in terms of analytical limits, data quality, reliability, costs, and operational feasibility. This evaluation will clarify any existing uncertainties and ensure the method is fit-for-purpose across diverse environmental contexts.

Method optimisation:
Based on results from experimental evaluation, the most feasible and robust methods will be further optimized. This includes improving selectivity, repeatability, accuracy, and overall robustness to finalize standardized testing protocols (SOPs). Optimization ensures consistent and high-quality data generation across laboratories.

Method validation:
The Joint Research Centre (JRC) will conduct targeted method validation to establish interlaboratory reproducibility and generate documentary guidelines. This step is vital to confirm the method's reliability irrespective of the testing laboratory and to provide performance benchmarks.

Stakeholder consultation:
Consultations with key expert stakeholders, including the Eionet AMR Working Group, the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR), and active Member States, will discuss the experimental outcomes. Topics will include sampling design, cost estimates, scalability, data management, result interpretation, communication strategies, and overall acceptability of the methodology. This engagement ensures transparency and broad consensus on next steps.

Drafting the implementing act:
Scientific and technical input from JRC based on the above stages will support the drafting of the legal implementing act, embedding the methodology within the EU framework.

1314 # List of abbreviations and definitions

Abbreviations

AI	Artificial intelligence
AMR	Antimicrobial resistance
AOP	Advanced oxidation processes
AQC	Analytical quality control
ARGs	Antibiotic resistance genes
ASTM	American Society for Testing and Materials
ATHINA	Advanced Technology for Health Intelligence and Action IT System
CARD	Comprehensive Antibiotic Resistance Database
CEN	European Committee for Standardisation (Comité Européen de Normalisation)
CFU	Colony forming unit
CHP	Combined heat and power
CLSI	Clinical and Laboratory Standards Institute
CPE	Carbapenemase-producing Enterobacterales
cpMLST	Core plasmid Multi-Locus Sequence Typing
ddPCR	Digital droplet Polymerase Chain Reaction
DG	Directorate-General
DG ENV	Directorate-General for Environment
DG JRC	Directorate-General Joint Research Centre
DNA	Deoxyribonucleic acid
dPCR	Digital Polymerase Chain Reaction
ECDC	European Centre for Disease Prevention and Control
EEA	European Environment Agency
EML List	WHO Model List of Essential Medicines
ENA	European Nucleotide Archive
ESBL	Extended-spectrum beta-lactamase
ESBL-Ec	ESBL-producing *Escherichia coli*
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU-JAMRAI	European Union Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections
EU-WISH	EU-Wastewater Integrated Surveillance for Public Health
FAO	Food and Agriculture Organization
FPKM	Fragments per Kilobase per Million reads
GLASS	Global Antimicrobial Resistance and Use Surveillance System
HaDEA	European Health and Digital Executive Agency
HERA	Health Emergency Preparedness and Response Authority
HGT	Horizontal gene transfer
HSC	Health Security Committee
HT	High throughput
ISO	International Organization for Standardisation
JA	Joint Action

MGEs	Mobile genetic elements
MIA List	WHO List of Medically Important Antimicrobials
MRSA	Methicillin-resistant *Staphylococcus aureus*
NGS	Next-Generation Sequencing
OIE	World Organisation for Animal Health
NUTS	Nomenclature of territorial units for statistics
PCR	Polymerase chain reaction
PE	Population equivalent
qPCR	quantitative Polymerase Chain Reaction
rRNA	Ribosomal ribonucleic acid
RT-qPCR	Reverse Transcription-quantitative Polymerase Chain Reaction
UN	United Nations
UV	Ultraviolet
WES	Wastewater and Environmental Surveillance
WGS	Whole genome sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health
WWTP	Wastewater treatment plant
2024 BPPL	WHO Bacterial Priority Pathogens List 2024

Definitions

Metagenomics	The study of the structure and function of entire nucleotide sequences isolated and analysed from all the organisms (typically microbes) in a bulk sample.
Shotgun metagenomics	A culture-independent technique for DNA sequencing technique used to analyse the genetic material of microbial communities.
16S rRNA gene sequencing	A method used to identify and classify bacteria and archaea by analysing the highly conservative 16S ribosomal RNA gene.
Metatranscriptomics	A method used to study the gene expression of microbial communities within specific environments by quantify all the mRNA transcripts produced by the microorganisms
Urban wastewater	means any of the following: (a) domestic wastewater; (b) the mixture of domestic wastewater and non-domestic wastewater; (c) the mixture of domestic wastewater and urban runoff; (d) the mixture of domestic wastewater, non-domestic wastewater and urban runoff
Agglomeration	'agglomeration' means an area where the population expressed in population equivalent, combined or not with economic activities, is sufficiently concentrated for urban wastewater to be collected and conducted to one or more urban wastewater treatment plants or to one or more final discharge points
1 population equivalent	"1 population equivalent" or '(1 p.e.)' means the organic biodegradable load per day, having a five-day biochemical oxygen demand (BOD5) of 60 g of oxygen per day;
Sludge'	'sludge' means organic and inorganic residue resulting from the treatment of urban wastewater from an urban wastewater treatment plant, excluding grit, grease, other debris and any other screenings and residues from the pre-treatment step;

One Health	'One Health' means One Health as defined in Article 3, point (7), of Regulation (EU) 2022/2371 of the European Parliament and of the Council

Annexes

Annex 1: Prompt template for data extraction

<u>Comprehensive Search Prompt:</u>

"Anti-microbial resistance monitoring in urban wastewater" AND "executive summary findings"

"Background and motivation for AMR research" OR "objectives and research questions in AMR studies"

"Methodologies for sampling protocols in wastewater"

a. "Overview of sampling protocols for AMR in wastewater"

b. "Strategies for sampling AMR in water environments"

c. "Types of environmental samples for AMR"

d. "Use of sampling equipment and techniques in AMR detection"

e. "Quality assurance and control in sampling protocols"

f. "Country-specific AMR sampling methodologies"

"Pre-treatment procedures in AMR testing"

a. "Overview of pre-treatment in AMR analysis"

b. "Filtration techniques for AMR samples"

c. "Chemical pre-treatment for AMR detection"

d. "Concentration methods for enhancing AMR detection"

e. "Alternative pre-treatment strategies in AMR analysis"

f. "Country-specific insights on pre-treatment methodologies"

"Detection methods for antimicrobial resistance in wastewater"

a. "Overview of detection methods in AMR research"

b. "PCR techniques in detecting AMR"

c. "Next-Generation Sequencing (NGS) for AMR"

d. "High-throughput screening methods for AMR detection"

e. "Bioinformatics and data analysis in AMR monitoring"

f. "Implementation of detection methods across countries"

"Data analysis in AMR studies"

a. "Statistical analysis of AMR data"

b. "Interpretation of AMR results and findings"

"Results on ARGs and antibiotic resistance"

a. "Overview of detected ARGs in various environments"

b. "Comparative analysis of AMR across different countries"

"Discussion on regional variations in antibiotic resistance genes (ARGs)"

a. "Implications of AMR variations for public health"

b. "Challenges and limitations in AMR research and detection"

"Conclusions on AMR research"

a. "Summary of key findings on AMR in wastewater"

b. "Future research directions in tackling AMR"

"Recommendations for policy and practice"

a. "Policy implications of AMR research findings"

b. "Strategic actions for monitoring and control of AMR"

"References and appendices in AMR research reports"

a. "Detailed tables and supplementary figures for AMR studies"

b. "Supplementary materials supporting AMR research"

<u>First prompt of national studies and specific applications:</u>

"Conduct a comprehensive review of the attached scientific publication focused on detecting antimicrobial resistance (AMR) in water and wastewater. Specifically, identify and list all mentioned antimicrobial resistances (AMR) and antibiotic resistance genes (ARGs) discussed in the papers. Examine:

Sampling Methods:

Detailed methodologies used for sampling water and wastewater for AMR analysis.

Techniques and equipment employed in the collection of samples.

Sample Pre-treatment:

Pre-treatment procedures applied to samples prior to AMR testing, including any filtration, chemical, or concentration methods.

Detection Methods:

Various detection techniques used for identifying AMR in water and wastewater.

Efficiency and applicability of techniques such as PCR, next-generation sequencing, and high-throughput screening.

Ensure the review highlights the standardised practices as well as any innovative or country-specific approaches discussed in the publications. List ALL AMR, ARGs and related parameters discussed in this publication and highlight specific aspects with regard to the aforementioned single analytical steps"

<u>Second prompt of national studies and specific applications:</u>

List all AMR and ARGs discussed in this publication

<u>Final prompt of national studies and specific applications:</u>

Considering the above provide now a comprehensive summary of the report.

Annex 2: Summary of extracted data from reviewed literature.

Table A1. Summary of extracted data for EU countries including references, sampling and analytical methods, targeted ARGs, standardisation status, and monitoring descriptors.

Country	Reference	Sampling and Analytical Methods	ARGs Monitored	Standardisation	Descriptors for Monitoring
Austria	(30,31)	**Sampling Methods**: Comparison of sampling schemes, such as grab samples and composite samples, including flow- and volume-proportional samples. **Analytical Methods**: qPCR, dPCR, HT-qPCR, shotgun metagenomics, and hybrid capture-based sequencing.	*sul1*, *ermB*, *tetW*, *vanA*, *blaTEM-1*, *nptII*, *nptIII.*	Emphasis on the development of standardised protocols and workflows for sampling, sample processing, molecular detection and bioinformatics analyses to ensure data reliability and inter-laboratory comparability. Focus aligns with the Urban Wastewater Treatment Directive (EU) 2024/3019 (Article 17(3)), WHO GLASS initiative and One Health frameworks.	Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL. Calculations of log reduction of ARG abundance between influent and effluent.
Belgium	(6,32)	**Sampling Methods**: Structured grab and composite sampling strategies from influent, effluent and receiving waters. Advocates for spatially distributed sampling. **Analytical Methods**: culture-based methods, complemented with ddPCR, qPCR and metagenomic sequencing.	*sul1, sul2, aadA, aac(3)-IV, blaCTX-M, blaNDM, blaSHV, blaKPC-2/3, ermB, ermF, qnrS, tetO, tetM, vanA.*	Focus on developing reproducible methodological workflows, harmonising molecular detection protocols and reporting formats to ensure standardisation across global research efforts. Calls for a multi-indicator approach aligned with the Urban Wastewater Treatment Directive (EU) 2024/3019 and the One Health strategy. No specific international standards are referenced.	Bacterial count as CFU per 100 mL of wastewater. Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL, per ng DNA, per day per PE. *intI1* included*:* marker for HGT and spread of ARGs. Calculations of log reduction of ARG abundance between influent and effluent.

Country	Reference	Sampling and Analytical Methods	ARGs Monitored	Standardisation	Descriptors for Monitoring
Bulgaria	(33,34)	**Sampling Methods**: Composite samples from influent and effluent points as well as from downstream of the WWTP discharge. **Analytical Methods**: Shotgun metagenomic sequencing.	*blaCTX-M*, *blaKPC-2/3*, *tetM*, *tetC, tetO, tetW, tetQ, tetX*, *ermB*, *ermF*, *mphE, msrE*, *vanA*, *sul1*, *sul2*, *aac(3)-IV, cfxA3, cfxA6*.	Emphasis on adopting standardised methodologies for consistent sampling, molecular detection, standardised bioinformatics pipelines and reproducible metagenomic workflows. Focus on benchmarking bacterial DNA extraction for metagenomic analysis. MetaCompare2.0 used to calculate the resistome risk score. No specific international standards are referenced.	*intI1* included*:* marker for HGT and spread of ARGs. Calculations of log fold change of bacterial taxa as a result of applied enrichment methods.
Finland	(35)	**Sampling Methods**: Sampling from influent untreated water. and since 2025 from treated wastewater effluents. **Analytical Methods**: culture-based enumeration, ddPCR, in-house qPCR, HT-qPCR, metagenomics.	*blaCTX-M*, *blaTEM, blaKPC-2/3*, *blaOXA, blaVIM*, *blaNDM*, *AmpC*, *mecA*, *tetM*, *ermB*, *vanA*, *sul2*, *aac(3)-IV*.	Importance of harmonising detection protocols for consistent results yet does not mention specific standards by international bodies. Stress metadata standardisation such as sample timing, concentration methods and analytical workflows. Report initiatives like the Global Sewage Surveillance Consortium. Participate in international AMR monitoring method development in ISO/TC 147/SC 4 "Microbiological methods" (Secretariat: DIN; Committee manager: Joana Gericke)	Bacterial count as CFU per 100 mL of wastewater. Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL, or total DNA per mL.

Country	**Reference**	**Sampling and Analytical Methods**	**ARGs Monitored**	**Standardisation**	**Descriptors for Monitoring**
France	(36–41)	**Sampling Methods**: Composite samples of influent and effluent urban and hospital wastewater, surface water, sludge, river, and groundwater sites using auto-samplers and manual collection. **Analytical Methods**: culture-based methods, HT-qPCR, 16S rRNA sequencing, and metatranscriptomics.	*blaRCP*, *blaLCR*, *blaFOX*, *blaNPS*, *blaTEM*, *aac(3)-Ib*/*aac(6')-Ib*, *aadB*, *aph(3'')*, *aph(6)*, *aac(3)-Iid*, *mph*, *catB3*, *ermF*, *vanA*, *tetQ*, *sul1*.	Propose a national framework within the One Health approach for harmonised methodology for ARG detection. Establishment of "AMR-Env" group, as part of the "PROMISE" project launched under the French Priority Research Program on Antimicrobial Resistance for standardised and harmonised collection and analysis methods in natural environments. Adhere to standardised protocols for sample pre-treatment, following ISO standards for measurement of micropollutants and ARGs. No specific mention of other international bodies like CEN or ASTM in the context of AMR in urban wastewater.	Concentration of antibiotic residues as ng/L Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL, copies per total DNA per mL or per bacterial cell. *intI1*, *intI2*, *intI3* included*:* markers for HGT and spread of ARGs. Include metadata descriptors, such as sampling date/location, methods used, normalisation procedures.
Germany	(13,42–92)	**Sampling Methods**: 24-hour composite and grab samples from urban, hospital and agricultural wastewater influents and effluents, surface waters, soils, sediments, biofilms, sludge, and drinking water reservoirs. **Analytical Methods**: culture-based methods, qPCR, dPCR, cultivation on selective agar media and subsequent WGS of isolates.	*blaTEM*, *blaCTX-M*, *blaOXA-48*, *blaVIM*, *blaNDM*, *blaKPC*, *sul1*, *tetM*, *ermB*, *mcr-1*, *vanA*.	Large-scale, multi-site and multi-country sampling efforts are undertaken to establish prevalence baselines. Recognise the need for standardised methodologies, but specific adherence to standards set by international bodies such as CEN, ISO, or ASTM is not mentioned. Have launched the NORMAN ARB&ARG database to promote data sharing, method alignment, and harmonised surveillance frameworks.	Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL. Presence of specific ARGs and AMR bacteria is used as indicators of pollution and potential health risks.

Country	Reference	Sampling and Analytical Methods	ARGs Monitored	Standardisation	Descriptors for Monitoring
Hungary	(93)	**Sampling Methods**: 24-h automated sampler composite influent wastewater samples. Grab samples from airport. **Analytical methods**: Culture-based methods, RT-qPCR with both commercially available and in-house methods.	*blaCTX-M1*, *blaNDM*, blaKPC, blaOXA-48, *vanA*, *vanB*.	Standardisation approaches are not discussed.	Absolute ARG gene copies per L of wastewater.
Ireland	(94–101)	**Sampling Methods**: Collection of influent and effluent wastewater from municipalities and hospitals. **Analytical Methods**: Culture-based detection, RT-PCR, cultivation followed by WGS.	*blaTEM*, *blaSHV*, *blaCTX-M*, *blaOXA-48*, *blaNDM-5*, *blaKPC-2*, *blaIMP*, *blaVIM*, *mcr-8*, *mcr-9*, *sul1*, *sul2*, *tetA,* aadA, cmlA.	Standardised guidelines such as those from EUCAST and CLSI are followed for antibiotic susceptibility testing. Recognise the need for systematic, standardised protocols to enable reliable national and transnational surveillance, and support One Health integration. No indication of adherence to standards set by international bodies such as CEN, ISO, or ASTM.	Bacterial count as CFU per 100 mL of wastewater. Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL. *intI1* included*:* marker for HGT and spread of ARGs. Include metadata descriptors, such as sampling date/location, methods used, and susceptibility thresholds.
Italy	(102–104)	**Sampling Methods**: Composite sampling over 24 hours. **Analytical Methods**: culture-based methods, dPCR, RT-qPCR.	*blaCTX-M*, *blaKPC*, *vanA*, *qnrS.*	Development of standardised protocols for AMR monitoring in wastewater through national level working groups. Protocols have been finalised. EUCAST clinical breakpoints used to define the resistance phenotypes and EUCAST algorithms for sensibility profiles.	Absolute ARG gene copies per mL of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL. *intI1* included*:* marker for HGT and spread of ARGs.

Country	Reference	Sampling and Analytical Methods	ARGs Monitored	Standardisation	Descriptors for Monitoring
Netherlands	(105–113)	**Sampling Methods**: Automatic 24-hour flow-proportional composite sampling from influent and effluent municipal and hospital wastewater. **Analytical Methods**: Culture-based methods, HT-qPCR, culturing followed by WGS, shotgun metagenomics.	*blaOXA-48*, *blaCTX-M*, *blaNDM*, *blaKPC*, *ermB*, *sul1*, *sul2*, *vanA*, *vanB*, *mcr-1*, *tetM*, *tetA*, *cmlA*, *qnrS*, *korB*.	Surveillance of AMR is based on consistent national protocols put forwards by the Dutch National Institute for Public Health and the Environment (RIVM) to support monitoring of longitudinal trends. The documents do not explicitly mention the involvement of international standardisation bodies such as CEN, ISO or ASTM.	Relative ARG abundance normalised to 16S rRNA gene copies per mL. Calculations of log reduction of ARG abundance between influent and effluent. *intI1* included*:* marker for HGT and spread of ARGs.
Portugal	(114–117)	**Sampling Methods**: 24-hour composite sampling and grab sampling from influents of WWTPs and hospitals. Deploy fractionated sampling. **Analytical Methods**: Culture-based isolation followed by antibiotic susceptibility testing (Kirby–Bauer disk method), dPCR, qPCR, shotgun metagenomic sequencing.	*blaTEM*, *blaSHV*, *blaCTX-M*, *blaCMY*, *mecA*, *vanA*, *vanB*, *mcr-1*.	Standardisation approaches are not discussed.	Bacterial count as CFU per 100 mL of wastewater. Absolute ARG gene copies per L of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL. Fractional quantification to distinguish ARG presence in bacteria versus phage DNA for vector transfer analysis.

Country	Reference	Sampling and Analytical Methods	ARGs Monitored	Standardisation	Descriptors for Monitoring
Sweden	(24,105,118–134)	**Sampling Methods**: Composite sampling over 24 hours using time-proportional and flow-proportional techniques from influent wastewater. **Analytical Methods**: Culture-base methods, qPCR, culturing followed by WGS, metagenomic sequencing.	*blaNDM*, *blaOXA-48*, *blaKPC*, *blaVIM*, *blaIMP*, *blaCTX-M*, *blaTEM*, *blaSHV*, *tetA*, *mcr-1*, *sul1*, *sul2*, *qnr* genes, *mphA*.	ARG annotations were harmonised using the CARD and ResFinder. The documents do not explicitly mention the involvement of international standardisation bodies such as CEN, ISO or ASTM.	Bacterial count as CFU per mL of wastewater. Absolute ARG gene copies per L of wastewater. Relative ARG abundance normalised to 16S rRNA gene copies per mL. Fragments per Kilobase per Million reads (FPKM) used to normalise library size in metagenomic analysis. Measure of unique ARGs per sample via Chao richness estimators.

Source: EC-JRC

Annex 3: National Obligations for AMR Monitoring in Aquatic Systems: EU-27 Comparative Analysis

Table A2. Overview of national obligations and monitoring practices for antimicrobial resistance (AMR) in aquatic systems across EU-27 member states. The dataset may not reflect all ongoing initiatives due to variability in national reporting and evolving legislations.

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Austria	No specific binding legal mandate for AMR-related water. Legally binding groundwater monitoring system (Water Rights Act 1959) on broad water quality parameters (such as pH, nitrates, heavy metals, pesticides)	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Net-work (ESAC-Net) 3. EU One Health Action Plan against AMR	1. National Action Plan for Antibiotics Resistance (NAP-AMR) 2. The PIER (Public Health Impact of Exposure to Antibiotic Resistance in Recreational Waters) 3. Infection Prevention and Control (PROHYG 2.0) 4. Antimicrobial Stewardship Programme (ASP)	1. AURES (Antibiotics Resistance and Antibiotics Usage) report – 2023 latest released 2. OECD report 3. ECDC report
Belgium	No specific binding legal mandate for AMR-related water. Monitoring in water is regionally implemented, guided by EU-level water and environmental directives	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Net-work (ESAC-Net) 3. European Antimicrobial Resistance Surveillance network in Veterinary medicine (**EARS-Vet**) 4. EU One Health Action Plan against AMR	1. National Action Plan AMR 2020-2024 2. "SanitelMed" data collection system for antimicrobial use in animals, based on legal obligations at the veterinary level 3. BEAST Projectv (Belgian Evaluation of Antimicrobial Stewardship Teams) 4. Surveillance Programs by Sciensano 5. Ongoing Development of New NAP AMR 2025-2029 6. IMHOTEP Project: Inventory of Hormonal and Organic Trace Matter in Heritage and Potable Waters	1. A 2023 study analysing recreational waters across Belgium found 24% of *E. coli* strains isolated from bathing sites resistant to at least one antibiotic, and 6% were multidrug-resistant. The Lesse river showed the highest levels of both absolute bacterial abundance and multidrug resistance (MAR index 0.063), with ESBL-producing *E. coli* present as well 2. The BELMAP report confirms robust national and regional AMR surveillance in humans and food animals, with MRSA prevalence falling but carbapenem-resistant *Klebsiella pneumoniae* and multidrug-resistant strains on the rise. 3. Belgium's hospitals largely follow EUCAST guidelinesv for susceptibility testing 4. OECD report 5. ECDC report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Bulgaria	No specific binding legal mandate for AMR-related water.	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Net-work (ESAC-Net) 3. EU One Health Action Plan against AMR	1. APUA Bulgaria Action plan to combat antimicrobial resistance in Bulgaria (2020-2024) 2. The Bulgarian National Reference Laboratory (AMR NRL) has been upgrading capacity for AMR testing, including accreditation and molecular diagnostics 3. Research and participation in EFFORT project, sponsored by EC 2013-2018	1. A two-year monitoring study from 2015, examined water samples from the Dam of Iskar and the Black Sea coastal zone. While this study may not have employed advanced metagenomic techniques, it laid groundwork for understanding the presence of antibiotic-resistant bacteria in Bulgarian water bodies 2. A 2024 metagenomic study of the Iskar River—Bulgaria's key freshwater resource—identified the presence of clinically relevant AMR genes (ARGs) such as carbapenemases (*blaOXA-58*, *blaIMP*) downstream of major wastewater treatment works (WWTPs), particularly after the Samokov plant. The study highlights anthropogenic impacts as a primary driver, with ARG levels typically rising closer to WWTP outlets 3. OECD report 4. ECDC report
Croatia	No specific binding legal mandate for AMR-related water. Institutions monitor environmental quality more generally but not specifically for AMR.	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Net-work (ESAC-Net) 3. European One Health Action Plan against AMR 4. NORMAN 5. AmReSu project 7. EU One Health Action Plan against AMR	1. National Control Program 2017-2021 2. The University Hospital for Infectious Diseases "Dr. Fran Mihaljevic" acts as Croatia's AMR reference center, supported by a network of public health laboratories; 3. Laboratories submit isolate data to the reference center for national analysis and reporting 4. ARES project	1. Several Croatian universities and public health institutions have conducted targeted AMR research in wastewater and river systems, identifying the presence of resistance genes (e.g., bla_TEM, bla_CTX-M) and multidrug-resistant E. coli in hospital effluents and municipal WWTPs.Bacterial count as CFU per 100 mL of wastewater 2. Ongoing research projects such as the ARES project are examining groundwater sources used for public water supply. This project employs advanced culture-independent methods (high-throughput qPCR, 16S rRNA sequencing) and culture-dependent analyses of clinically important pathogens (e.g., *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumanii*) isolated from groundwater 3. OECD report 4. ECDC report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Cyprus	No specific binding legal mandate for AMR-related water. The detection of AMR in environmental samples in Cyprus has been limited to academic and EU-funded projects, such as surveys of antibiotic-resistant bacteria in treated wastewater and coastal waters.	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) 4. European Union Reference Laboratory for Public Health on AMR (EURL-PH-AMR) 5. EU One Health Action Plan against AMR	1. National Action Plan (NAP) on AMR (2023–2027); 2. Cyprus, as a water-scarce country, has an advanced wastewater reuse system mainly for irrigation and groundwater enrichment.	1. Studies such as a 2020 investigation into treated effluent reuse for irrigation identified the presence of resistance genes (e.g., sul1, qnrS, bla_TEM) in samples from wastewater treatment plants 2. 2025 Danube Water Conference: Optimizing water reuse to address water scarcity in Cyprus 3. Community Antibiotic Consumption in Cyprus for the Period 2015 to 2022 4. OECD report 5. ECDC report
Czechia	No specific binding legal mandate for AMR-related water. The environmental AMR data in Czechia is gathered exclusively through academic or state-funded pilot studies, not through binding national legislation.	1. NORMAN 2. AMR Watch project 3. European Antimicrobial Resistance Surveillance Network (EARS-Net) 4. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 5. Joint Programming Initiative on AMR (JPIAMR)	1. National Antibiotic Programme (NAP); 2. A National Reference Laboratory (NRL) for antibiotics leads surveillance activities and offers reference services, coordinating a network of local laboratories. 3. Ongoing development includes strategies extending toward 2030, addressing AMR in the aquatic environment and enhancing One Health approaches. 4. Additional initiatives include public projects like the Prevention of Antibiotic Resistance project, aimed at reducing antibiotic use in the population.	1. A 2022–2023 national study on surface waters and WWTP effluents in Czechia revealed that treated wastewater discharged into rivers contained E. coli isolates resistant to ampicillin, ciprofloxacin, gentamicin, and third- to fourth-generation cephalosporins. Many isolates were also ESBL-producers and carried class 1 integrons, indicating high potential for resistance gene transfer. 2. The issue of antimicrobial resistance in the aquatic environment of the Czech Republic 3. OECD report 4. ECDC report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Denmark	No specific binding legal mandate for AMR-related water. Efforts are part of research programs and sectoral stewardship initiatives.	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. Joint Programming Initiative on AMR (JPIAMR) 4. World Health Organisations (WHOs) programme for surveillance of antimicrobial consumption and resistance, GLASS	1. National Action Plan (June 2025) 2. Danish Integrated Antimicrobial Monitoring and Research Program - Antimicrobial Consumption and Resistance Surveillance Unit (DANMAP) 3. International Centre for Antimicrobial Resistance Solutions (ICARS)	1. Green Procurement Guidelines published by Amgros (a national pharmaceutical procurement agency) in 2023 require pharmaceutical companies to declare "environmentally harmful medicines", including antibiotics 2. The Danish Environmental Protection Agency (EPA) funded a 2022 study showing the presence of sulfonamides, macrolides, and fluoroquinolones in effluent and surface waters 3. On July 3, 2025 the government has released a Guidance on water quality and supervision of water supply facilities 4. National initiatives use metagenomic surveillance of wastewater to monitor AMR trends. A study of Danish WWTPs reported AMR genes (ARGs) related to tetracycline, sulfonamide, and beta-lactam resistance found in influent and effluent waters 5. OECD report 6. ECDC report
Estonia	No specific binding legal mandate for AMR-related water.	1. Joint Programming Initiative on AMR (JPIAMR) 2. European Joint Action on AMR (EU-JAMRAI) 3. NORMAN	1. AMR Management Strategy 2025–2030	1. Environmental surveillance of AMR in Estonia's aquatic systems is currently limited but evolving. Regional data suggest that like other Baltic Sea region countries, Estonia's aquatic environments may serve as hotspots for AMR gene dissemination due to human activity, though systematic studies remain limited 2. ECDC report 3. OECD report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Finland	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Net-work (ESAC-Net)	1 National Action Plan 2024–2028 2. Finnish institutions such as the Finnish Institute for Health and Welfare (THL) and the Finnish Environment Institute (SYKE) are participating in projects like TruSTme (2023–2024), which explores wastewater treatment plants as real-time surveillance tools for AMR threat, including analysis of antibiotic resistance genes and microplastics in effluent 3. FINRES-Vet	1. A study found a high diversity of ARGs especially in non-disinfected systems; disinfected water systems had higher ratios of ARG reads relative to bacterial markers and elevated MRGs (mercury, arsenic resistance) suggesting selective pressures in treated waters. 2. Focus on pharmaceuticals in water 3. Finland has carried out hospital wastewater monitoring studies in Helsinki hospital effluents, weekly sampling detected and quantified multiple antibiotic resistance genes (ARGs), including carbapenem resistance genes blaGES, blaVIM, blaKPC, and others using HT-qPCR and digital platforms 4. University of Helsinki research on antimicrobial drugs repurposing 5. ECDC report 6. OECD report
France	No specific binding legal mandate for AMR-related water	1. European Joint Action on AMR (EU-JAMRAI)	1. 2022-2025 National Strategy for Preventing Infections and Antibiotic Resistance 2. Interministerial Roadmap for 2024-2034 was published recently that sets a longer-term vision involving multiple ministerial plans in human and animal health sectors with 17 strategic objectives around awareness, research, surveillance, stewardship, innovation, and international cooperation	1. ANSES report on antibiotic resistance and antibiotics in the environment 2. Studies highlight hospital wastewater as a significant hotspot for AMR bacteria such as Pseudomonas, Acinetobacter, and Klebsiella pneumoniae, with ARGs being detected through municipal wastewater treatment stages, though advanced treatment like UV disinfection reduces final effluent contamination 5. ECDC report 6. OECD report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Germany	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. EU One Health Action Plan against AMR	1. "DART 2030" (German Antimicrobial Resistance Strategy) 2. SARA (Surveillance of Pathogens and Antibiotic Resistances in Aquatic Ecosystems) project 3. The *RAaaO* project (at TU Darmstadt testing ultrasound-ozonation combinations to reduce ARGs in wastewater treatment and evaluating molecular detection across resistance gene spectra)	1. National wastewater surveillance is undertaken by the *Robert Koch Institute (RKI),* collaborating with the Umweltbundesamt and other federal and regional bodies. Ongoing monitoring includes antibiotic resistance genes (e.g., carbapenem-resistance in Enterobacterales) in sewage treatment plant effluents, using culture-based, digital PCR, and metagenomic methods 2. Study on fish resistomes and WWTP impacts 3. Holtemme river study 4. ECDC report 5. OECD report
Greece	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. European Joint Action on AMR (EU-JAMRAI)	1. National Strategy for Quality of Care and Patient Safety for Greece 2025–2030	1. A 2023 four-year longitudinal study of a major WWTP in Northern Greece (Thessaloniki) found persistent levels of macrolides and fluoroquinolones (e.g. azithromycin, clarithromycin, ciprofloxacin) in influent and treated effluent. Concentrations rose during the COVID-19 pandemic and posed risks for AMR selection in receiving waters. 2. Environmental isolates of *Pseudomonas aeruginosa* collected from various Greek aquatic environments, rivers and coastal areas, included numerous ESBL-producers and isolates with class 1 integrons, indicating that Greek surface waters can host resistant strains 3. In Athens's Psyttalia WWTP (serving ~4.6 million people), a 2025 study assessed the removal efficiency of both conventional activated sludge and nature-based treatment systems (e.g. constructed wetlands plus UV disinfection) for antibiotic-resistant bacteria (ARB) and resistance genes (ARGs). The findings highlight significant persistence of ARGs post-treatment 4. Research on antibiotic resistance in Lake Karla, Thessaly (2024) 5. Study on wastewater treatment challenges for ARG removal in Greece 6. ECDC report 7. OECD report

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Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Hungary	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. EU One Health Action Plan against AMR 4. European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI 2) 5. JPIAMR/OHAMR Research Roadmap	National antimicrobial resistance (AMR) strategy is currently in development or draft form for the period 2025–2029	1. A 2023 study reported high levels of pharmaceuticals including ibuprofen, naproxen, diclofenac, and carbamazepine in surface water of the Danube in Pest County. These compounds were detected despite standard water treatment, pointing to environmental persistence and potential AMR selection pressure 2. Shotgun metagenomic sequencing on untreated sewage from North Pest WWTP (Budapest) in 2016 found diverse antibiotic resistance genes (tetracycline, aminoglycoside, β-lactamase) overlapping with those found in animals and sludge, indicating environmental dissemination routes via wastewater and sludge reuse 3.Bibliometric analysis of antibiotic resistance in aquaculture emphasizing Hungary's research contributions and trends 4. ECDC report 5. OECD report
Ireland	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. JPIAMR/OHAMR Research Roadmap 4. European Joint Action on AMR (EU-JAMRAI)	1. National AMR Action Plan iNAP2 (2021–2025) 2. HSE Antimicrobial Resistance Infection Control (AMRIC) Action Plan 2022–2025	1. A 2023 study investigating public and private drinking water supplies in Ireland identified Enterobacterales including *E. coli*, *Serratia*, and *Enterobacter* with resistance to amoxicillin (~55%) and ciprofloxacin (<10%) highlighting potential AMR reservoirs in water supplies 2. Another investigation involving wastewater and surface water sampling between 2019 and 2020 identified 419 Enterobacterales isolates. Notably, 78% of water and 50% of sewage samples contained extended-spectrum beta-lactamase (ESBL) producers, with carbapenemase genes (e.g. bla_OXA-48, bla_NDM, bla_KPC) detected at multiple sites 3. The PIER (Public Health Impact of Exposure to Antibiotic Resistance in Recreational Waters) project and EPA-supported studies have highlighted the presence and persistence of AMR bacteria in recreational and drinking water, reinforcing the need for environmental AMR surveillance 4. ECDC report 5. OECD report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Italy	No specific binding legal mandate for AMR-related water	1. European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI 2) 2. European Antimicrobial Resistance Surveillance Network (EARS-Net) 3. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 4. JPIAMR/OHAMR Research Roadmap	National Action Plan on Antimicrobial Resistance 2022–2025 (PNCAR)	1. A study monitoring wastewater treatment plants (WWTPs) in Sicily (Pantano D'Arci, Siracusa, Giarre) detected several ARGs such as blaSHV, erm(B), blaOXA, blaTEM, and blaCTX-M persistently in treated wastewater, indicating that aquatic systems serve as persistent AMR gene reservoirs potentially impacting public health 2. Large-scale aquatic AMR monitoring by Italian Military Navy (article) collecting aquatic AMR data across multiple marine and freshwater sites, contributing to a growing dataset on aquatic resistomes and the environmental prevalence of ARGs 3. ECDC report 4. OECD report
Latvia	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. Transatlantic Taskforce on AMR (TATFAR) 4. European Joint Action on AMR (EU-JAMRAI) 5. JPIAMR/OHAMR Research Roadmap	1. One Health AMR Action Plan 2023-2027 2. LATOHOP's (EC supported project)	1. Monitoring for bacteria in rivers, lakes and ponds was conducted in 2001 and 2002 but there is no data available on further analyses 2. ECDC report 3. OECD report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Lithuania	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. EU One Health Action Plan against AMR 4. Transatlantic Taskforce on AMR (TATFAR) 5. European Joint Action on AMR (EU-JAMRAI) 6. JPIAMR/OHAMR Research Roadmap	National Action Plan on Antimicrobial Resistance (PNCAR)	1. Lithuania has conducted studies on the microbiome and resistome dynamics across a salinity gradient, analysing water and sediment samples from the Baltic Sea coast and the Curonian Lagoon between 2017 and 2023. These studies aimed to examine the microbiome and resistome dynamics, providing insights into the diversity of antibiotic resistance genes in aquatic environments 2. ECDC report 3. OECD report
Luxembourg	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. EU One Health Action Plan against AMR 4. Transatlantic Taskforce on AMR (TATFAR) 5. European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI 2) 6. JPIAMR/OHAMR Research Roadmap 7. Transatlantic Taskforce on AMR (TATFAR)	National Action Plan on Antimicrobial Resistance (PNCAR) 2018-2022 which was extended until 2024. Concrete details on a 2025 extension or the launch of a new national plan for 2025 onwards remain unavailable as of now	1. Luxembourg has been involved in research projects studying the prevalence and spread of antimicrobial resistance, including the monitoring of antibiotic-resistant bacteria in various environments. These studies contribute to understanding the scope of AMR but are not part of a legally mandated surveillance system 2. In 2024, Luxembourg hosted the 2nd WES-LUXEMBOURG DAY, focusing on the growing challenge of antimicrobial resistance (AMR). The event showcased how wastewater and environmental surveillance can help detect and track resistance genes and resistant pathogens at both national and European levels 3. ECDC report 4. OECD report

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Malta	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. EU One Health Action Plan against AMR 4. Transatlantic Taskforce on AMR (TATFAR) 5. European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI 2) 6. JPIAMR/OHAMR Research Roadmap 7. Transatlantic Taskforce on AMR (TATFAR)	1. National Strategy & Action Plan for the Prevention and Containment of Antimicrobial Resistance (2020–2028). The Strategy will be regularly reviewed and updated so that it remains reflective of the work that is underway, and action plans for the future. The review Dates are set for 2023 and 2026; 2. WHO-documented national AMR strategy covering 2020–2028	1. Addressing AMR in Malta, National AMR Committee presentation 2. ECDC report 3. OECD report
The Netherlands	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) 3. EU One Health Action Plan against AMR 4. European Joint Action on AMR (EU-JAMRAI) 5. JPIAMR/OHAMR Research Roadmap	1. National Action Plan on Antimicrobial Resistance (AMR) for 2024–2030 2. "Chain Approach" (2016 – ongoing) 3. AMR3 (2022–2025)	1. Continuous monitoring and reporting of antibiotic use and resistance trends in humans and animals through the annual NethMap/MARAN report (2024) 2. The Dutch chain approach *on pharmaceuticals in water: Stakeholders acting together to reduce the environmental impact of pharmaceuticals* 3. ECDC report 4. OECD report

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Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Poland	No specific binding legal mandate for AMR-related water	1. JPIAMR/OHAMR Research Roadmap 2. EU One Health Action Plan against AMR	1. National Health Programme 2021–2025; 2. BINWIT platform at the Polish Academy of Sciences, enhancing access to scientific resources relevant to bacteriophages and immunology	A 2020 study found that ~70% of monitored antimicrobial agents including ciprofloxacin, macrolides, and sulfamethoxazole were present in influent and effluent of two Polish wastewater treatment plants. The concentrations posed medium to high risk for resistance selection in receiving rivers, even after conventional treatment (~50% removal efficacy) 2. A 2020 study identified antibiotic resistance genes (ARGs) in wastewater systems, including hospital effluents, underscoring the dissemination of resistant determinants via treatment facilities 3. A research conducted in Oder river water shows microplastic particles in water environments may serve as vectors for dispersing pathogens and ARGs 4. Recent Polish study on AMR in recreational lakes (2025) 5. Study on microbial shifts post-flooding in Wrocław (2025) 6. ECDC report 7. OECD report
Portugal	No specific binding legal mandate for AMR-related water	1. European Antimicrobial Resistance Surveillance Network (EARS-Net) 2. European Surveillance of Antimicrobial Consumption Net-work (ESAC-Net) 3. EU One Health Action Plan against AMR 4. JPIAMR/OHAMR Research Roadmap	1. National plan on AMR covers the period around 2019–2023/24, with ongoing engagement and updates to align with EU and WHO guidelines 2. "Drive-AMS" training course, which by early 2025 had expanded to involve sixteen national hospitals aiming to improve antibiotic prescription practices and reduce antibiotic-resistant bacteria emergence	1. A 2025 study analysing environmental samples from 65 sheep and goat farms in central Portugal, detected antimicrobial resistance genes (ARGs) associated with multiple antibiotic classes including β-lactams, tetracyclines, macrolides, and sulfonamides in water, soil, pasture, and bedding. ARGs were found in 83% of samples, many exhibiting multidrug resistance potential 2. ECDC report 3. OECD report

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Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Romania	No specific binding legal mandate for AMR-related water	1. EU One Health Action Plan against AMR 2. JPIAMR/OHAMR Research Roadmap	1. National Recovery and Resilience Plan (NRRP) 2. Romania and Norway collaborate against Antibiotic Resistance	1. A 2024 study assessed antibiotic residues and microbial loads in three Romanian wastewater treatment plants (WWTPs) between 2021 and 2022. It documented persistent antibiotics including amoxicillin and clarithromycin in effluent waters, posing medium to high environmental risk and potential for resistance selection even after treatment. 2. Comprehensive one Health–oriented research highlights the presence and transmission of resistant bacterial strains and antibiotic resistance genes (ARGs) in wastewater influent and effluent. For example, Klebsiella pneumoniae ST101, including carbapenem-resistant isolates, was detected throughout two Romanian hospitals co-presenting resistance and heteroresistance to colistin. 3. Study of Romania's high antibiotic use and AMR challenges (2025). 4. Qualitative study on antibiotic treatment and prevention challenges in Romania (2024) 5. Recent Romanian research on Enterobacterales and AMR in Aquatic Environments (2025) 6. Review on Heavy Metals and Microplastics Impacting AMR in Aquatic Systems 7. ECDC report 8. OECD report
Slovakia	No specific binding legal mandate for AMR-related water	1. EU One Health Action Plan against AMR 2. JPIAMR/OHAMR Research Roadmap 3. European Antimicrobial Resistance Surveillance Network (EARS-Net)	National Action Plan on Antimicrobial Resistance for the period 2019–2021; formally published distinct national plan explicitly titled for the year 2025 is not clearly documented in available public sources.	1. A pilot study conducted at two wastewater treatment plants in Bratislava and Košice measured concentrations of 33 antibiotics, including ciprofloxacin and clarithromycin, in influent and effluent. Removal efficiencies were insufficient, and resistant fecal coliforms were also detected in sludge. Sub-inhibitory antibiotic levels create selective pressure for resistance development. 2. A 2023 study of Acinetobacter spp. in the WWTP at Kokšov-Bakša (Košice) identified genetically diverse, antibiotic-resistant strains—indicating the plant as a potential environmental reservoir and contributor to AMR spread in receiving waters. 3. Another year-long study in Petržalka WWTP (Bratislava) documented multidrug-resistant coliforms and enterococci in both influent and effluent, including E. coli and Morganella morganii. 4. ECDC report 5. OECD report

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Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Slovenia	No specific binding legal mandate for AMR-related water	1. EU One Health Action Plan against AMR 2. JPIAMR/OHAMR Research Roadmap 3. EU JAMRAI 2 - JAZMP	Current national strategy covers 2019–2024, with a draft for the new National "One Health" AMR Strategy planned for 2025–2030	1. A pilot study in Slovenia (2007) investigated removal of pharmaceutical residues including ibuprofen, diclofenac, ketoprofen in a pilot wastewater treatment plant. The study showed variable removal efficiencies, with certain compounds persisting post-treatment, highlighting the limitations of conventional WWTPs in eliminating pharmaceutical pollutants 2. Slovenia's Waters Act was amended in 2021, but the amendment was overwhelmingly rejected via referendum due to concerns about weakening water protection. There is no national legislation mandating AMR or antibiotic residue monitoring in surface or wastewater 3. ECDC report 4. OECD report
Spain	No specific binding legal mandate for AMR-related water	1. EU One Health Action Plan against AMR 2. JPIAMR/OHAMR Research Roadmap 3. European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI 2)	Spain's national strategy on antimicrobial resistance (AMR) for 2025 is framed within the ongoing Spanish National Action Plan against AMR (PRAN), which was originally launched in 2014. The existing Water Law (Ley de Aguas, 1985) delegates water quality regulation to basin agencies but does not require AMR or antibiotic residue monitoring.	1. PRAN coordinated by AEMPS and MITECO in collaboration with IDAEA-CSIC, published a *Report 2.2* (2024) monitoring five antibiotics including erythromycin, clarithromycin, azithromycin, ciprofloxacin, and amoxicillin in 16 WWTPs and associated rivers. Elevated residues were found throughout, confirming environmental exposure and potential risk for AMR selection. 2. A recent study (2024) assessed pharmaceutical residues in Catalonia's main rivers (Llobregat and Besòs), analysing 78 pharmaceuticals using LC–MS/MS. Detectable levels of ciprofloxacin, macrolides, NSAIDs, and others were found, highlighting pharmaceutical contamination hotspots in surface waters. **3.** A 2023 urban study from Northern Spain measured culturable antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in influent and effluent of two WWTPs. Findings included ESBL-producing *E. coli* and diverse ARGs persisting after treatment suggesting WWTP effluents as ongoing sources of AMR dissemination. 4. A pilot study in Castellón used wastewater-based epidemiology (WBE) during 2021–22 to quantify 11 antibiotics and multiple ARGs concurrently; azithromycin and ciprofloxacin were prevalent, with strong correlations observed between overall antibiotic load and the intl1 resistance gene 5. Recent review on micromotor nanotechnology for AMR remediation in Spanish water environments (2025) 6. Seasonal ARG and antibiotic residue study in Spanish Mediterranean waters

Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
				7. ECDC report 8. OECD report

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Country	National Obligation	EU Linkage	National Projects / Instruments	Additional References
Sweden	Binding legal requirement (Water Management Ordinance HVMFS 2019:25)	1. EU One Health Action Plan against AMR 2. JPIAMR/OHAMR Research Roadmap 3. European Antimicrobial Resistance Surveillance Network (EARS-Net)	1. Swedish Strategy to Combat Antibiotic Resistance was adopted in February 2020 and extended in November 2023 to run until the end of 2025; 2. CARe - Centre for Antibiotic Resistance Research in Gothenburg is a collaborative and interdiscplinar research centre at the University of Gothenburg and Chalmers University of Technology that conducts research into the environmental dimensions of antibiotic resistance 3. SLU Future One Health (slu.se) platform for interdisciplinary research and collaborations that provides knowledge and solutions to good in sustainable ecosystems 4. STRAMA, the strategic program against antibiotic resistance, which provides education and monitors resistance trends	1. National *Environmental Quality Standards* (EQS) for certain antibiotics such as ciprofloxacin (0.1 µg/L, MAC-EQS) classified as river basin-specific pollutant; AMR relevance explicitly acknowledged 2. A strong cross-sectoral national collaborative function led jointly by the Public Health Agency of Sweden and the Swedish Board of Agriculture, which engages 21 government agencies and 5 organizations to ensure a coordinated response across sectors 3. Antibiotic Smart Sweden's Criteria for Municipal Wastewater 4. The Swedish experience - a summary on the Swedish efforts towards a low and prudent use of antibiotics in animal production (In 1986, Sweden, as the first country in the world banned all use of antibiotics as growth promoters in food animal production) 5. Sweden's successful approach to antibiotic resistance can be used as inspiration in other countries – 2023 – ReAct 6. ECDC report 7. OECD report

Source: JRC

Note on the source:

European Environmental Agency. Urban Waste Water Treatment Directive, Treatment plants reported under UWWTD data call 2021 - PUBLIC VERSION, Jan. 2023.

UWWTD Treatment Plants, Jan. 2023 is one of the datasets produced within the frame of the reporting under 12th UWWTD Art.15 reporting period (UWWTD data call 2021). The Urban Waste Water Treatment Directive (UWWTD) (91/271/EEC) obliges Member States to report data on the implementation of the Directive upon request from the European Commission bi-annually. Reported data include receiving areas as designated under UWWTD, agglomerations, urban wastewater treatment plants serving the agglomerations and points of discharges. Dataset UWWTD Treatment Plants contains urban wastewater treatment plants and collecting systems without UWWTP, including their coordinates, capacity and actual load treated, type of treatment and data on the performance of plants. This dataset includes the reported treatment plants displayed on the UWWTD maps (https://www.eea.europa.eu/themes/water/european-waters/water-use-and-environmental-pressures/uwwtd/interactive-maps/urban-waste-water-treatment-maps-3).

The active and connected treatment plants with correct coordinates in the reported data were selected from the source European UWWTD tabular dataset which is available on the download link https://www.eea.europa.eu/data-and-maps/data/waterbase-uwwtd-urban-waste-water-treatment-directive-9. The definition of the UWWTD Treatment Plants dataset attributes (fields) is available on the link:. The full dataset, including non active and non connected treatment plants are available under "Urban Waste Water Treatment Directive, Treatment plants reported under UWWTD data call 2022 - INTERNAL VERSION, Jan. 2023" (https://sdi.eea.europa.eu/catalogue/inspire-pds/eng/catalog.search#/metadata/0d0c9029-735e-4968-a577-9c22fcf7b2b0). The published output contains data reported in 2022. Current output is provisional, as it is subject to the Commission's compliance check, following which some records may be amended, and further information will be added.

Annex 5: Cost estimation and evaluation for monitoring AMR in wastewater

Sampling

Monitoring AMR in WWTPs is typically conducted at the plant's inlet and outlet. This process is often integrated with existing sampling activities aimed at process control or compliance checks, which assess various biological and chemical parameters such as chemical pollutants, *E. coli*, and other pathogens. To capture the dynamics of AMR effectively and avoid missing peak occurrences, it is advisable to use time or flow-integrated sampling methods. These methods involve collecting representative samples over a period or based on flow volume, and they benefit from the efficiency and consistency provided by automated equipment. Larger WWTPs often already utilise such equipment, so the cost estimates provided below should be considered in the context of implementing a new automated system, rather than the significantly lower cost of incorporating additional sampling into an existing workflow.

Table A5. Cost overview for sampling equipment needed for AMR monitoring. The figures are based on actual offers (year of reference 2024) and JRC based experiences

Cost type	Cost for	Description	Estimate
Capital costs	Automated Sampling Equipment	Sampler Purchase depending on features such as refrigeration, portability, and data logging capabilities	€3,000 to €10,000
		Auxiliary Equipment Includes items like flow meters and additional infrastructure or mounting apparatus	€1,000 to €5,000,
Operating costs	Maintenance and Calibration	Regular Maintenance: Routine checks and servicing to ensure equipment accuracy and longevity	€500 to €1,000 per year
		Calibration: Accurate measurement requires periodic calibration, typically annually	€100 to €300
	Consumables	Bottles and Tubing: Replacement of sampling bottles and tubing due to wear or contamination	€200 to €500 annually.
		If samples require preservation for later analysis, chemicals might add a minor expense	€50-€200 per year
	Data Management and Connectivity	Software and Subscription Systems: Some automated samplers offer data logging or remote connectivity features that might require software licenses or subscriptions	€100 to €500 annually.
Labor Costs	Setup and Operation	Installation Labor: Setting up the system involves initial installation	€500 to €2,000 for contractor services, plus training.
		Ongoing Monitoring: Personnel time for monitoring equipment performance, resolving issues, and retrieving samples	€1,000 to €3,000 annually depending on the frequency of sample collection and analysis

Source: EC-JRC

Cost for PCR measurements

a) ‘Conventional’ quantitative PCR (qPCR)

The cost of a qPCR test for AMR can vary widely depending on several factors, including whether it's performed in-house or sent to an external lab, the specific genes being targeted, and the number of samples. Generally, costs range from around 50 to 1000 Euro per test. To illustrate, in-house testing using a kit like the LuminUltra GeneCount® Q-16 qPCR system, the cost can be as low as 42 Euro per test, while sending samples to an external lab can cost between 300 and 1,000 Euro per test. Specific kits such as for instance AMR1 multiplexed qPCR assay bundles from pcrassays.com can cost around 550,000 Euro. The complexity of the assay, the number of genes being targeted, and the type of reagents used can all influence the final cost. In addition to the direct testing cost, it's important to consider the cost of specialised equipment like qPCR machines, which can range from hundreds of thousands to millions of dollars for cutting-edge last generation technology.

Hence, estimating the cost of qPCR test for ARGs in wastewater is complex and involves considering various factors including reagents, equipment, labour, and overhead (Table A6). In the following is a breakdown based on JRC internal experiences and recently collect quotations in the context of the so-called European Super-Sites Sentinel System for Wastewater.

Given these factors, the total cost for processing a single sample through qPCR for AMR/ARGs in wastewater can vary significantly, typically ranging from 50 to 400 Euro per sample depending on economies of scale, location, and institutional pricing agreements. For a more precise estimate, each component would need to be itemised and adjusted based on specific laboratory settings, sample throughput, and geographic considerations.

Table A6. Cost overview for AMR monitoring by qPCR. The figures are based on actual offers (year of reference 2024) and JRC based experiences

Cost type	Cost for	Description	Estimate
Material and Equipment	Reagents and Consumables	Custom primers and probes specific to the AMR/ARG targets depending on the complexity and supplier	€100 to €500 per assay
		high-quality q-PCR master mix	€1 to €5 per reaction
		The consumables for running reactions, including plates and seals	€0.50 to €1 per reaction
		Nucleic Acid Extraction Kits depending on the kit's efficiency and supplier	from €2 to €10
	Equipment	qPCR Machine depending on its capacity and features. The depreciation costs over several years can be factored into the cost per test.	€10,000 to €100,000
	Miscellaneous Lab Equipment	Include costs for centrifuges, pipettes, and other necessary equipment, though these are not typically broken down per test	
Labour	Sample Collection and Processing	Personnel costs for collecting samples and preparing depending on location and expertise	€20 to €50 per hour of labour
		Running and analysing qPCR involves setting up the reactions, running the qPCR, and analysing the data	1-3 hours of skilled labour
Overhead and Indirect Costs	Facility Costs	Rental, utility, and maintenance costs for the laboratory space	*Not quantified*
	Quality Control and Reporting	Includes expenses related to ensuring reliability of results and preparing reports.	*Not quantified*
Additional Costs	Research and Development	If the assay requires the development of new primers/probes or testing protocols.	*Not quantified*
	Compliance and Accreditation	Adhering to regulatory standards may incur additional costs	*Not quantified*

Source: EC-JRC

b) Digital droplet PCR (ddPCR) cost evaluation

ddPCR is an advanced nucleic acid detection method that offers greater precision and sensitivity compared to quantitative PCR (qPCR). Unlike qPCR, which monitors DNA amplification in real-time to quantify DNA based on the fluorescence emitted throughout the reaction and typically requires a standard curve for absolute quantification, ddPCR partitions the sample into thousands of nanolitre-sized droplets, conducting PCR in each droplet. This partitioning allows for absolute quantification by directly counting the proportion of droplets containing the target DNA, eliminating the need for a standard curve and offering excellent resistance to sample inhibitors. This makes ddPCR especially suitable for detecting low-abundance targets, such as rare alleles, low-level viral loads, and minimal residual disease in cancer monitoring, providing a high level of precision that is crucial in clinical diagnostics and cutting-edge research. However, ddPCR is generally lower in throughput compared to qPCR and is generally more expensive than qPCR due to several factors. First, ddPCR requires specialised equipment for droplet generation and partitioning, which tends to have a higher initial cost compared to standard qPCR machines. Additionally, the consumables for ddPCR, including reagents and droplet generation materials, can be more costly. This results in a higher per-reaction cost for ddPCR compared to qPCR. While ddPCR offers superior sensitivity and precision, especially for detecting low-abundance targets or rare mutations, the increased cost may be a consideration for large-scale studies or routine analyses where the enhanced sensitivity of ddPCR is not necessary.

Despite these higher costs, the benefits of ddPCR in terms of accuracy and robustness against inhibitors may justify the expense in clinical diagnostics and specialised research applications. A more detailed cost-breakdown is displayed in the table below.

Table A7. Cost overview for AMR monitoring by ddPCR. The figures are based on actual offers (year of reference 2024) and JRC based experiences

Cost type	Cost for	Description	Estimate
Material and Equipment	Reagents and Consumables	Custom primers and probes specific to the AMR/ARG targets depending on the complexity and supplier. Similar to qPCR, but the specific reagents for partitioning and droplet generation can be more expensive.	€100 to €500 per assay
		Droplet Generation and Master Mixes	€5 to €10 per reaction
		The consumables for running reactions, including plates and seals, etc.	€2.50 to €10 per reaction
	Equipment	ddPCR Machine depending on its capacity and features. The depreciation costs over several years can be factored into the cost per test.	€50,000 to €200,000
	Miscellaneous Lab Equipment	Include costs for centrifuges, pipettes, and other necessary equipment, though these are not typically broken down per test	
Labor	Sample Collection and Processing	Personnel costs for collecting samples and preparing depending on location and expertise	€20 to €50 per hour of labour
		Running and analysing ddPCR involves setting up the reactions, running the qPCR, and analysing the data	1-3 hours of skilled labour
Overall costs		Cost per ddPCR test for AMR/ARGs	€100 - €400

Source: EC-JRC

c) Cultivation-based PCR cost evaluation

Cultivation-based PCR, unlike standard PCR, begins with an initial step where microorganisms are grown in specific culture media before PCR amplification. While this process aims to enrich and isolate viable organisms for analysis, confirming microbial viability and providing insights into active populations, it has notable drawbacks. The method is significantly more time-intensive and resource-demanding due to its reliance on incubation periods and specific growth conditions, leading to slower results compared to the rapid efficiency of qPCR. Additionally, cultivation-based PCR can introduce bias, as some microorganisms do not grow well in laboratory settings, potentially skewing results and underrepresenting certain species. In contrast, qPCR bypasses these limitations by quickly detecting genetic material from both viable and non-viable organisms, offering more comprehensive and timely data. For many applications, especially those requiring high throughput and quick turnaround, qPCR is favoured due to its speed, lower cost, and the ability to provide immediate insights without the prolonged and selective steps inherent in cultivation-based methods. A6 gives an overview on the costs for cultivation-based PCR Methods.

Table A8. Cost overview for AMR monitoring by cultivation-based PCR. The figures are based on actual offers (year of reference 2024) and JRC based experiences

Cost type	Cost for	Description	Estimate
Direct costs	Culture Media and Materials	The cultivation step requires media, cost of which depends on the organism and media complexity.	€1 to €5 per assay
		Cultivation Time and Space. Considerations include incubator use and lab space, adding a cost per sample due to time and resource allocation	€5 to €10 per reaction
Follow-up costs[1]	Reagents and Consumables	for the DNA extraction and amplification steps	€5 to €15 per sample
Labour	Extended Time for Growth	Cultivation significantly extends the timeline for obtaining results, impacting labour costs, depending on the duration and complexity of thorough microbial culturing.	€50 to €100
Overall costs		Cost per Cultivation-based PCR test for AMR/ARGs	€100 - €300

[1] for equipment etc. see Table A5

Source: EC-JRC

d) Costs for metagenomics

Conducting a cost evaluation for metagenomics specifically targeting AMR/ARGs involves multiple components that contribute to the overall expense. Below is a breakdown similar to what was used for qPCR but adapting to the unique requirements of metagenomic analysis.

Table A9. Cost overview for AMR monitoring by metagenomics. The figures are based on actual offers (year of reference 2024) and JRC based experiences

Cost type	Cost for	Description	Estimate
Sample collection and preparation	Collection and Storage	Similar to other methods, for collection and cold transport.	€5 to €20 per sample
	DNA Extraction	High-quality extraction kits or automated systems are crucial for metagenomics	€5 to €20 per sample
Library Preparation	Kits and Reagents	Preparing DNA libraries for sequencing is a significant cost, depending on the kit and protocol used	€50 to €150 per sample
Sequencing cost	Platform and Throughput	Costs vary based on the chosen sequencing platform (e.g., Illumina, Oxford Nanopore) and the depth of sequencing required. Costs depend on the desired coverage and data output.	€100 and €1,000 per sample. Higher costs are possible.
Bioinformatics Analysis	Hardware and Software	Initial setup might involve costs for computational hardware or cloud resources, alongside software licenses or subscriptions for specialised analysis tools.	€50 to €300 per analysis
	Expert Analysis	Interpretation of metagenomic data requires specialised bioinformatic expertise, costs depending on complexity and depth of analysis.	€100 to €300 per sample
Overhead and Indirect Costs	Personnel and Training	Skilled personnel are needed to manage and conduct metagenomic workflows	€200 to €500 per project
	Facility and Equipment Maintenance	Includes depreciation and maintenance of sequencing equipment and laboratory facilities.	Not quantified
Overall costs	Per Sample Costs	Cost per metagenomics for AMR/ARGs	€400 - €3500
	Economies of Scale	Larger batch sizes can reduce costs per sample due to more efficient use of sequencing platforms and shared bioinformatics resources.	

Source: EC-JRC

Metagenomics stands out for its agnostic approach to sequencing, offering unparalleled value in the study of AMR and ARGs Unlike targeted methods such as qPCR, which require prior knowledge of specific genes, metagenomics allows for the comprehensive profiling of entire microbial communities. This means that all genetic material present in a sample is sequenced and analysed, making it possible to capture the full resistome, including known and previously unidentified resistance genes. This holistic perspective is crucial for understanding the complexity and dynamics of resistance in diverse environments, especially in settings where unknown or emerging resistance mechanisms may be present.

Indeed, one of the most significant advantages of metagenomics is its ability to create a digitalised snapshot, or "memory," of the genetic content within a sample. The data generated acts not only as a current analytical tool but also as a rich resource that can be revisited as new insights or technologies emerge. This allows researchers to re-analyse the sequenced data at a later stage, making metagenomics a forward-thinking investment that can adapt to future scientific questions. For instance, if new ARGs are discovered or if there are advancements in bioinformatics, existing metagenomic datasets can be re-mined for additional insights, providing immense long-term value.

This ability to store and re-assess data enhances the strategic importance of metagenomics, justifying its higher initial costs. While it is significantly more expensive than qPCR, metagenomics provides depth and breadth that are unmatched by more targeted methods. For decision-makers, the choice between qPCR and metagenomics should weigh the study's objectives, budget constraints, and the potential need for comprehensive or retrospective analyses. For routine surveillance or studies focusing on specific genes, qPCR's cost-effectiveness and speed make it an attractive choice. However, in research that requires expansive data collection, the identification of novel resistance genes, or when dealing with complex, multi-pathogen communities, metagenomics offers unparalleled insight, making it a valuable tool despite its higher cost.

Emerging technologies: Lab-on-a-Chip and Smart-Chips

The field of AMR monitoring is witnessing a technological evolution with the introduction of innovative platforms such as lab-on-a-chip sensors and Smart-Chip technologies. These cutting-edge solutions offer the promise of transforming AMR surveillance through a decentralised and cost-effective approach.

These emerging technologies, now entering increasingly also the European market, promise to democratise access to AMR monitoring, enabling widespread adoption in various geographic and resource settings. They are particularly advantageous for remote or under-resourced locations, where traditional laboratory setups may be impractical. By facilitating quick, on-site AMR detection, these devices can enhance decision-making processes for patient treatment or environmental intervention, potentially improving health outcomes and reducing the spread of resistance.

Despite their potential, lab-on-a-chip and smart-chip technologies are at an early stage of market deployment. There are challenges related to mass manufacturing, device standardisation, and the robustness of sensors, which must be addressed to ensure reliability and consistent performance. While these devices can generate large volumes of data, integrating this information into existing health and environmental data systems remains a challenge. Effective data handling, storage, and interpretation infrastructure need to be established. Ensuring these technologies meet stringent regulatory standards for diagnostic accuracy and reliability is crucial for widespread adoption, particularly in clinical settings.

a) Lab-on-a-Chip sensors:

These microfluidic devices integrate multiple laboratory functions onto a single chip, capable of handling small fluid volumes. They can efficiently perform sample preparation, DNA amplification, and detection of AMR genes, effectively miniaturising and automating complex laboratory processes. By reducing the need for bulky equipment and labour-intensive procedures, lab-on-a-chip devices significantly cut down costs associated with traditional AMR detection methods. They enable rapid, point-of-care testing, which can be particularly beneficial in settings lacking extensive laboratory infrastructure.

b) Smart-Chip technologies:

Smart-Chips incorporate advanced biosensors and electronic components, potentially allowing real-time data monitoring and transmission. This makes them suitable for continuous monitoring applications, where data can be instantly analysed and shared via digital networks. These chips facilitate a shift towards more localised monitoring of AMR, improving responsiveness and enabling targeted interventions in both healthcare and environmental contexts. Their deployment could significantly reduce sample transport and processing costs.

Cost comparison and potential savings

When comparing the potential costs of emerging technologies like lab-on-a-chip sensors and smart-chip platforms to a traditional PCR cost of approximately 250 Euro per sample, these innovative platforms can offer significant savings over time.

Initially, Lab-on-a-Chip and Smart-Chip Per-Test Costs per test using might be somewhat comparable to or slightly less than traditional PCR, possibly ranging from 50 to 150 Euro per test depending on the complexity of the test and initial deployment costs. As production scales up and technology advances, costs could potentially fall below 50 Euro per test.

As these technologies mature and manufacturing scales up, the cost per test is likely to decrease significantly. Mass production can dramatically lower the cost of materials and components, pushing costs well below traditional PCR estimates. By providing on-site, rapid testing and reducing logistics and processing times associated with centralised laboratories, these technologies can cut down operational costs related to sample transport, handling, and lab personnel. Once deployed, the automated nature of these devices minimises the need for extensive laboratory infrastructure and manpower, potentially leading to lower operational expenses over time. The potential to reconfigure and update these devices for new tests without high additional costs further enhances their cost-effectiveness, offering continual savings compared to re-developing specific PCR tests.

While initial costs for deploying lab-on-a-chip sensors and smart-chip technologies may be higher or comparable to traditional PCR testing, they hold the potential to significantly reduce per-sample costs in the long run. With costs that could eventually fall well below 50 Euro per test as the technology matures, these innovations promise substantial savings. This potential reduction in cost aligns with a decentralised and efficient approach to AMR monitoring, especially as the market and technology evolve.

The emergence of lab-on-a-chip sensors and smart-chips marks a pivotal shift towards more accessible, efficient, and potentially low-cost AMR monitoring. While these technologies hold significant promise, ongoing advancements and overcoming current limitations will be essential to fully realise their potential and enhance the global capacity to monitor and respond to antimicrobial resistance effectively. Their integration could complement existing technologies like qPCR and metagenomics, providing a comprehensive suite of tools to tackle AMR on multiple fronts.

Market insights from public procurement

In 2024, the European Commission's HaDEA published a EU4Health call for tenders HADEA/2024/OP/0017 - Framework contracts to provide services and supplies to measure pathogens and pollutants of emerging concern in untreated wastewater samples as part of a global sentinel system.

This call aimed at purchasing supplies and equipment for wastewater sampling and testing, and services to measure pathogens and pollutants of emerging concern in untreated wastewater samples at strategic locations known as super-sites. These super-sites will constitute a sentinel system which will be part of the global sentinel system that will ensure exchange of data to establish an early warning system for future pandemics. In this context, price information for measurements of pathogens by PCR, WGS/NGS measurements and Shotgun Metagenomics in untreated wastewater samples were obtained. The information collected allowed a comprehensive assessment of market prices for some AMR/ARG targets and was further confirmed by own surveys and tenders performed by the JRC.

The observed and document price range for the measurement of azole resistant Aspergillus fumigatus, Carbapenem-/colistin-resistant Enterobacterales, Carbapenem-resistant / colistin- resistant *Acinetobacter baumannii*, Carbapenem-resistant *Pseudomonas aeruginosa* and other emerging MDR or XDR pathogens including MRSA ranged from 30 – 200 Euro per sample and parameter employing different PCR techniques, with an average of ca. 85 Euro per sample and parameter.

Prices for untargeted ('shotgun') sequencing of all ('meta-') microbial genomes present in untreated wastewater samples (metagenomics) ranged from 300 to 4700 Euro with an average cost of ca. 1950 Euro per sample.

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