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Antimicrobial Resistance and Healthcare-Associated Infections

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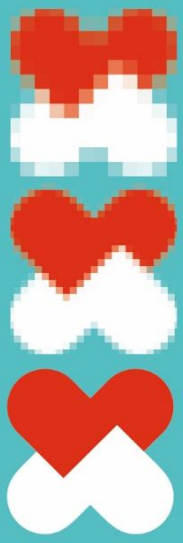
EU-JAMRAI 2 – Milestone 17

Drafting a common EARS-Env approach for environmental surveillance of AMR



BUILDING A ONE HEALTH WORLD ♥
to reduce Antimicrobial Resistance (AMR)

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1 ACRONYMS

3GCR 3rd Generation Cephalosporin-resistant

AMR Antimicrobial Resistance

ARB Antibiotic-resistant Bacteria

ARGs Antibiotic Resistance Genes

COVID-19 Coronavirus disease 2019

CPE Carbapenemase-producing Enterobacterales

CRE Carbapenem-resistant Enterobacterales

DNA Deoxyribonucleic acid

dPCR Digital Polymerase Chain Reaction

ESBL Extended Spectrum Beta- Lactam

EU-JAMRAI-2 EU Joint Action on Antimicrobial Resistance and Healthcare Associated Infections 2

EU-WISH EU Joint Action on Wastewater Integrated Surveillance in Health

HT-PCR High-Throughput PCR

PCR Polymerase Chain Reaction

qPCR Quantitative Polymerase Chain Reaction

UWTTD Urban Wastewater Treatment Directive

WBE Wastewater-Based Epidemiology

WBS Wastewater-Based Surveillance

WES Wastewater and Environmental Surveillance

WGS Whole-Genome Sequencing

WHO World Health Organisation

WWTP Wastewater Treatment Plant

2 EXECUTIVE SUMMARY

THE COMMON EARS-ENV APPROACH: PRIORITIZED CORE ELEMENTS FOR ENVIRONMENTAL AMR SURVEILLANCE

'The environment' can have many different roles in AMR dynamics and the environmental dimension of AMR is a rapidly developing field. In contrast to surveillance of AMR in the human and animal sector, there is little international harmonization of surveillance of AMR in the environment (Schwermer et al., 2025). Therefore, establishing a common approach consisting of prioritized core elements of environmental AMR surveillance is one of the tasks of EU-JAMRAI-2. This includes establishing prioritized objectives of environmental surveillance. Such objectives are fourfold: environmental surveillance can shed light on the emergence of new AMR mechanisms; It can also help generating risk assesment data to inform the attribution of AMR in humans and animals resulting from environmental exposures; moreover, it can provide information on the quality of the environment, trends of AMR in the environment, or, for wastewater-based surveillance, on trends of AMR circulating in the human population; last, environmental surveillance can help evaluate the impact of interventions and to assess the resilience capacities of an environment under stress.

Based on the results of a survey, several prioritization workshops, and discussions that have taken place among EU-JAMRAI-2 members working in environmental surveillance, core elements for environmental AMR surveillance have been prioritized and agreed on as of November 2025. The core elements for environmental AMR surveillance consist of a combination of surveillance objectives, environmental domains and surveillance targets and are outlined below.

These core elements are suggested as a common approach to environmental AMR surveillance across Europe, for countries initializing environmental surveillance at national, regional, or local level. Such a common approach across Europe can help compare countries, and address cross-

border contamination with AMR. These core elements should be considered as common minimum priority actions for AMR surveillance, but not as an exhaustive approach – countries can expand these core elements where needed based on local priorities.

While we have included a wide range of objectives, compartments, and AMR targets in our considerations, we cannot rule out that the exact expertise of the consulted collaborators and participants might have influenced the prioritization. Future discussions will shed light on topics that have been underrepresented in the specific group of experts consulted.

Last, given the rapid development of AMR and the emergence of new resistance mechanisms, the results of such prioritization exercises might change in future. A prioritization of at least AMR targets should be repeated regularly (such as every few years).

AMR surveillance in surface waters

The first priority for environmental surveillance consists of surveillance of AMR in surface water, serving the following objectives:

1. To collect data that can contribute to human risk assessment of the presence of AMR in the environment, and
2. To collect data that provides information on spatiotemporal trends of AMR in the environment.

For both objectives the following two AMR targets are prioritized:

- 3rd generation cephalosporin-resistant (3GCR) *E. coli* detected by culture, followed by phenotypic characterisation.
- A panel of resistance genes, including at least relevant beta-lactam resistance gene panels, detected by PCR (dPCR, qPCR), in the total microbial community DNA.

Definitions and further specifications:

- Surface water can include both natural and artificial waterways, and in this common approach, groundwater is also regarded to be connected with surface water. Surface water locations with specific purposes can be considered, including recreational water, drinking water sources**,** or irrigation water. A more in-depth specification of which surface water locations should be prioritized for sampling will be one of the future EU-JAMRAI-2 activities including a pilot.
- Risk assessment is regarded here as analyses of the extent of human and animal exposure to AMR in the environment, ultimately serving to shed light on the attribution of the carriage of resistant bacteria or infections with resistant bacteria to environmental exposures.
- Analysis of resistant bacteria is regarded here as analysis through cultivation, followed by phenotypic characterization (antibiotic susceptibility testing). Whole-genome sequencing of isolates can aid in attributing resistance circulating in animals and humans to environmental exposures.

- With respect to resistance genes, specific targets for PCR-based detection will be identified in the near future, including at least beta-lactam resistance genes such as the *bla*$_{CTX-M}$ group.
- The recommendation includes two targets momentarily (a culture-based and a PCR-based target). A choice for one of those targets might be made in future, also based on experiences gained in the pilot, and based on the viewpoints collected in consultations with other sectors.

AMR surveillance in wastewater

Wastewater surveillance is considered as a special case within environmental surveillance. Wastewater-based surveillance generally conducted in raw wastewater of wastewater treatment plants (WWTP) is seen as a complement to public health monitoring of the human population. Effluents of WWTP are a source of bacteria and resistance genes for aquatic environments. Since wastewater monitoring will become mandatory according to Directive (EU) 2024/3019, we decided to prioritise objectives and targets for wastewater separately from objectives and targets from other environmental compartments. Prioritisation for wastewater has (partly) been conducted in collaboration with the EU Joint Action on Wastewater Integrated Surveillance in Health (EU-WISH).

The first priority within wastewater surveillance i is to monitor AMR in raw municipal wastewater, serving the following objectives:

1. To determine temporal AMR trends of AMR circulating in humans
2. To detect emerging mechanisms of AMR.

The following targets are prioritized to address the first objective:

- carbapenem-resistant Enterobacterales (CRE) detected by cultivation,
- a panel of resistance genes, including at least relevant carbapenem and beta-lactam resistance genes, detected in the total microbial community DNA by PCR (dPCR, qPCR).

The following targets are prioritized to address the second objective:

- Resistance genes in the total microbial community by metagenomic analyses

A second priority is to monitor treated wastewater (in addition to raw wastewater), to 1) assess the efficacy of the treatment processes and 2) estimate the discharge of AMR into the environment with wastewater.

In order to evaluate the efficacy of wastewater treatment and the emissions from WWTPs to surface water, one common target, an indicator of process effectiveness, should be tested in raw and treated wastewater as well as surface water. A suitable target would consist 3GCR *E. coli*.

Definitions and further specifications

- Emerging mechanisms of AMR are regarded here as new types of phenotypic resistance that have not or rarely been reported (Iera *et al*. 2025), and new genetic determinants of AMR that may have high potential for spread.
- Analysis of resistant Enterobacterales is regarded here as analysis through cultivation in selective media, followed by phenotypic characterization (antibiotic susceptibility testing). Whole-genome sequencing (WGS) of isolates can aid in detecting specific genotypes of CPE.
- Specific targets for PCR-based detection will be identified in the near future, including carbapenem resistance genes such as *bla*$_{NDM}$ and *bla*$_{KPC}$, and extended spectrum beta-lactam(ESBL) resistance genes such as those within the *bla*$_{CTX-M}$ group.
- The recommendation for the first objective includes two targets momentarily (a culture-based and a PCR-based target). A choice for one of those targets might be made in future, also based on experiences gained in the planned pilot, and based on the viewpoints collected in consultations with other sectors.

Local adaptations and other additional elements

Local adaptations

We have prioritized one to three common objectives, compartments and AMR targets but recognize the need and the value of additional local adaptations to combine local and overarching goals of environmental surveillance. We recommend a similar approach for prioritization of surveillance objectives, compartments and AMR targets with local experts and stakeholders.

Additional elements

Soil represents an additional environmental compartment for environmental surveillance which can be considered by countries , with the objective of determining trends of AMR in the environment or to determine the efficiency of interventions (such as manure treatment). Priority targets have not yet been set and require further discussions.

Surveillance of crops and vegetables can generate data that can be fed into risk assessment and is closely linked to the soil domain. However, crops and vegetables are generally considered to fall in the domain of AMR surveillance in food – here, the exact distribution of surveillance between the environmental and food domain requires to be determined per country.

Other compartments, such as wildlife, soil microfauna, plants, plastisphere, air/dust, etc., have increasingly been identified as contributors to AMR. Ongoing research continues to reveal additional compartments that may influence AMR dynamics, and further candidates are likely to emerge. These could be considered for inclusion in future phases or local adaptations of environmental surveillance. This also holds for drinking water sources, or hospital wastewater.

Next to the already mentioned prioritized targets per surveillance objective, the following AMR targets were repeatedly mentioned during meetings and prioritisation exercises. We mention them here to serve as inspiration for local or future adaptations.

- Fluoroquinolone resistance genes
- Carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*
- Fluoroquinolone-resistant Salmonella
- *Vibrio* spp
- *Aspergillus fumigatus*

3 INTRODUCTION

3.1 Aim of the task

This document describes Milestone 17 of Jamrai workpackage 8: a common approach for environmental surveillance. The milestone is the result of the work performed in subtask 8.3.1 "Collect information from countries on existing environmental surveillance and its goals, and specify goals and associated targets for a common environmental surveillance."

Since 'environmental surveillance' is a broad field with many possible directions (including wastewater surveillance), it is necessary to prioritize which compartments, surveillance objectives, and targets should be addressed. The common approach is our advice on these priorities for individual countries and for Europe as a whole that wish to take steps towards environmental AMR surveillance.

3.2 Short background

'The environment' can have many different roles in AMR dynamics, and it is important to recognize that the environmental dimension of AMR is a rapidly developing field. The environment can act as recipient and reservoir of AMR, but also as a source of new resistance mechanisms, as well as a transmission route, facilitating the spread of resistant bacteria to humans, animals or food (UNEP 2023). Although the knowledge on the exact nature and extent of these roles is still limited, a call for environmental surveillance has clearly been made (EU council 2023, UN General Assembly 2024).

In addition, the environment is a broad concept that can be defined in multiple ways. In the context of AMR, and specifically within EU-JAMRAI-2, the focus is primarily on water and soil, as these two environmental compartments are the most extensively studied with respect to AMR, while research on the dynamics of AMR in air is still in its early stages. These two main compartments consist of many sub-compartments such as surface water, ground water, agricultural soils, urban soils, etc.

Wastewater is also often considered an environmental compartment, as are, to a lesser extent, the effluents from healthcare institutions. Other compartments, such as wildlife, soil microfauna, plants, plastisphere, air, etc., have increasingly been identified as contributors to AMR.

Ongoing research continues to reveal additional compartments related to or included in 'the environment' that may influence AMR dynamics, and further candidates are likely to emerge. Furthermore, challenges and data gaps exist with respect to the environmental fate of pathogens, AMR determinants, antibiotic residues and metals, and the role of climate change, such as temperature increases, torrential rains, or changes in salinity. The current knowledge gaps with

respect to AMR in the environment can be addressed by research studies. In addition, surveillance activities can also play a role in addressing the environmental aspects of AMR.

Surveillance can serve different objectives. Environmental surveillance can shed light on the emergence of new AMR mechanisms. It can help generate data to inform the attribution of AMR in humans and animals resulting from environmental exposures to AMR (for assessing transmission-related risks). One third objective consists of determining temporal or spatial trends in the presence of AMR in the environment. Specifically, wastewater-based epidemiology (WBE) or wastewater-based surveillance (WBS), which is increasingly being used, can inform on AMR circulating in the human population. While wastewater is often considered to fall within the umbrella of environmental surveillance, wastewater based surveillance therefore rather represents a surveillance activity complementary primarily to human health surveillance. Last, environmental surveillance can help evaluate the impact of interventions and to assess the resilience capacities of an environment under stress. Objectives, approaches and research needs for environmental surveillance have been addressed (Larsson and Flach 2022, Bengtsson-Palme *et al*. 2023), yet, few internationally harmonized approaches or protocols (sampling, analysis, DNA-extraction data treatment) have been presented (Schwermer *et al*. 2025).

Given the complexity of the environment, its numerous compartments and diverse matrices, and the wide range of possible surveillance objectives, prioritizing the development of environmental surveillance is essential. Such prioritization will help ensure that limited resources and capacity are directed toward the most impactful efforts. This exercise should start with a clear definition of the surveillance objective – the rationale or the 'why' behind the activities, followed by the selection of the most relevant environmental compartment and precise AMR target to be measured.

3.3 What gap do we fill?

A major gap currently exists in Europe due to the fragmentation of approaches to environmental AMR surveillance, the lack of harmonised priorities and methodologies. With our work in 8.3 we evaluate common grounds across Europe on core elements of environmental AMR surveillance, harmonized amongst the participants of EU-JAMRAI-2 (and where relevant, with participants of EU WISH). We also build capacity at governmental institutions in the members states on environmental surveillance.

3.4 EU-WISH

The Joint Action EU-WISH is designed to prioritize and design future actions in wastewater surveillance, and a part of their work covers AMR. Since the aims of EU-JAMRAI-2 and EU-WISH with respect to establishing an approach for surveillance of AMR in wastewater largely overlap, an official collaboration has been established in February 2025. This entails that, regarding AMR wastewater surveillance priorities and related activities (e.g., protocols), EU WISH and EU-JAMRAI-2 closely collaborate. Moreover, the EU recast of the urban wastewater treatment directive (UWTTD) entails that AMR surveillance in wastewater will become mandatory.

Therefore, we decided to prioritise objectives and targets for wastewater separately from objectives and targets from other environmental compartments.

4 STEPS TAKEN TOWARDS DEFINING CORE ELEMENTS OF ENVIRONMENTAL AMR SURVEILLANCE

The strategy for developing a common approach involved two surveys, several workshops, and a cross-check with experts from other One Health domains. This approach entails comparable steps as used before for prioritizing AMR surveillance in the water environment (Liguori *et al.* 2022). As a result, a 'current' common approach has been established, part of which will be tested through a pilot study. The final common approach, to be proposed by EU-JAMRAI-2 in 2027, will be an optimized version that incorporates the results of the pilot as well as relevant scientific and other developments that may arise.

As mentioned before, 'environmental surveillance' is a broad field with many possible directions. It is therefore necessary to prioritize which compartments, surveillance objectives, and AMR targets should be addressed. As a principle for both the survey analyses and the prioritization workshops, the following core steps were followed to come to priorities for the future of environmental surveillance:

1. Decision on the highest-priority surveillance objective and environmental compartment
2. Decision on the highest-priority sub-objective
3. Subsequent decision on the most important characteristics of AMR targets to be measured, as well as on the specific AMR targets of choice.

The main sources for the options discussed in the abovementioned steps included:

- Surveillance objectives: Draft Quadripartite report on integrated surveillance, Larsson and Flach (2022), Bengtsson-Palme *et al.* (2023)
- AMR target characteristics: Draft WHO report on wastewater and environmental surveillance for one or more pathogens (2024)
- National pathogen surveillance priorities and relevant scientific literature (potential targets)

4.1 The survey

A survey was developed during the third and fourth quarters of 2024 to gather information from countries of the 8.3 WP and experts about their current environmental surveillance activities and their perspectives on priorities for future surveillance. The questions in the survey were based on

scientific literature, expert knowledge, and consultation within the EU-JAMRAI-2 WP8.3 group. The survey was distributed in the first quarter of 2025, and countries were given approximately 6 months to respond. EUSurvey software was used to create the survey.

The questions addressed topics such as the selection of environmental compartments for surveillance, surveillance objectives, methods, AMR targets, sampling strategies, and potential barriers to implementation (links to the survey can be found in the annex).

The terminology used to refer to environmental (sub)compartments was that of the EU legislation, whenever applicable. In the case of soil systems, the land cover and land use ontology from EU Copernicus programme was used. The language of the survey was English only.

Survey 1 – Past & Present

This survey aimed to map existing environmental AMR monitoring activities across different environmental compartments (e.g., wastewater, surface water, soil). It is referred to as the "Past & Present" survey in the following document.

Survey 2 – Future

This survey assessed surveillance objectives, sampling compartments, and AMR targets considered desirable for future environmental AMR surveillance. It is referred to as the "Future" survey in the following document.

Definition of a Surveillance System

In order to guide the collection of info on existing environmental AMR surveillance activities, a surveillance system was defined as a structured approach targeting one or more environmental compartments (e.g., wastewater, surface water, soil), with a defined selection of sampling sites, sampling frequencies, and targets such as specific resistant bacteria and/or resistance genes. Examples include:

- Ongoing surveillance conducted under existing legal frameworks
- Repeated surveillance of environmental compartments not covered by current legislation
- Pilot or research-based surveillance, conducted either once or repeatedly, by governmental institutions, research organizations, or universities

Activities at local, regional, or national scales were eligible for inclusion. We also encouraged reporting surveillance work performed by research organizations when environmental surveillance represented a key objective of the project.

This broad inclusion strategy was chosen because we expected few ongoing national-level surveillance systems, but were aware of many research projects and pilot studies contributing to methodological development. We also recognized that some countries apply different approaches at the regional level. A complete list of all environmental AMR research activities is unattainable; therefore, national experts participating in EU-JAMRAI-2 were asked to identify the most relevant studies in their country.

Survey Participation

Both questionnaires targeted experts involved in environmental surveillance. Respondents were asked to focus on surveillance systems within their domain of expertise, and collaboration with colleagues was encouraged to ensure complete and accurate responses.

The call for participation was distributed by email to national points of contact and to a broader network of European experts. The Future survey was also disseminated in social media (Linkedin).

4.2 Workshops on prioritization

Parallel to the survey, online workshops on the group members' vision on priorities for future environmental surveillance were organized in Q2 2025. These were both conducted internally (within the 8.3 group) and externally (in a joint workshop between EU-JAMRAI-2 WP8.3.1 and the WGAMR2 group of Joint Action EU-WISH). An external global meeting was also held at the watermicro2025 conference in Amersfoort.

During or before these workshops, a short version of the survey was worked through, and results were discussed during the workshop. The questions addressed which environmental compartment and surveillance (sub) objective the members would prioritize, followed by specifying essential characteristics of associated priority AMR targets, and finally, selecting specific AMR targets. The exact questions were inspired by current literature addressing possible objectives of environmental and WBS, as well as by the draft WHO guidance on WES.

4.3 Cross check with other one health domains (human and animal)

In order to guarantee inclusivity and sustainability of the proposed common approach, a series of One Health cross check events have started to incorporate viewpoints from other domains. A first cross-check was conducted internally within EU-JAMRAI-2 WP8 across all WP tasks and One Health sectors and, thereafter, cross checks with relevant European human and animal health institutions and agencies and an even broader EU-JAMRAI-2 audience will take place. In these events, especially those aspects of the common approach aiming at increasing public and animal health are critically discussed.

4.4 Workshops for decision making

During the EU WISH prioritization workshop in Dublin in September 2025, the common approach for wastewater surveillance was discussed, adjusted, and agreed on. During the EU-JAMRAI-2 environmental workshop in Madrid in October 2025 the common approach for wastewater surveillance and environmental surveillance was further discussed and agreed on.

5 OUTCOME OF SURVEYS AND PRIORITISATION WORKSHOPS

5.1 Summary of prioritization workshops

In the first **EU-JAMRAI-2/ EU WISH workshop**, using Mentimeter, the first and second prioritized objective/compartment combinations consisted of

- **Determining AMR trends in humans in raw wastewater** (32/54 votes), with the associated AMR targets (ranked by frequency of occurrence): carbapenem-resistant Enterobacterales (15/97 target mentions), *E. coli* (10/97) and carbapenem resistance genes (10/97). Overall, the great majority of participants voted for analysis of specific resistant or generic bacteria by culture (73/97), while 15 voted for analysis of resistance genes by PCR and 7 for metagenomic analyses.
- **Determining emerging forms of AMR in raw wastewater** (19/51 votes), with metagenomic analyses as priority target (26/76 target mentions). Overall, more participants voted for analysis of specific resistant or generic bacteria by culture (35/76) than for metagenomic analyses and PCR-based analyses.

Sub-objectives mentioned included determination of the sources of AMR in the environment, determination of temporal variation, and identification of hotspots of emergence and evolution of AMR for the second objective. One comment of the participants was that the process should be repeated, specifically addressing environmental surveillance next to wastewater surveillance – due to the fact that no specific objectives addressing environmental surveillance had been prioritized by the participants.

One subsequent **EU-JAMRAI-2 workshop** therefore specifically addressed environmental surveillance (i.e., surveillance other than WBS in WWTPs).

- The main objectives mentioned across all environmental compartments were to generate data contributing to risk assessment (18/32) and to determine AMR trends (11/32) – emergence of AMR was not mentioned.
- The environmental compartment prioritized by most participants across all objectives was surface water (10/20 as first objective resp. 13/32 in 1st and 2nd objective together). Recreational water was mentioned 9/32 times as first or 2nd objective. Among all AMR targets suggested for surface and recreational water, 3GCR *E. coli* (or ESBL producing *E. coli*) (14/41) was mentioned most frequently, with genes and/or metagenomes (9/41) and CRE (8/41) also mentioned as priority targets.
- For raw wastewater, most participants prioritized the determination of AMR trends (13/17) with temporal variation as sub-objective. As AMR targets, CRE (7/25), 3GCR *E. coli* and metagenomic analyses (7/25) were mentioned most often as priority targets.

One subsequent **EU WISH workshop** (6/6/25) specifically addressed WBS (i.e., surveillance in raw and treated wastewater).

- The main objectives mentioned for raw wastewater were to determine temporal trends in AMR, and to study emergence of new clinically relevant AMR mechanisms. Overall, for trends, most participants voted for analysis of carbapenem-resistant Enterobacterales by culture (10/32) with some (6) also advising to use metagenomic analyses. For emerging mechanisms of resistance, metagenomic analyses were voted most often (10/22).
- The main objectives mentioned for treated wastewater were to generate data for risk assessment (and specifically for environmental exposure) as well as to study interventions (i.e., efficiency of wastewater treatment). There was no clear agreement with respect to the best AMR targets for these objectives – specific resistant but also non-resistant bacteria were mentioned next to metagenomic analyses. For treatment efficiency, most participants suggested to study carbapenem resistance genes.

Last, one **workshop at the watermicro2025 conference** in Amersfoort (attracting about 200 participants) was held in June 25. Here, mentimeter was used.

- The main objectives mentioned for raw wastewater were to determine temporal trends in AMR in humans (57/108), followed by studying emergence of new clinically relevant AMR mechanisms (19/108) and efficiency of interventions (16/108). Overall, for trends on AMR, most participants voted for analysis of carbapenem-resistant Enterobacterales by culture (20/108) with 14/108 also advising to use metagenomic analyses (a considerable proportion answered "I don't know" or "I do not have the capacity to decide" (34/108), possibly reflecting participants not considering themselves experts in AMR (89/112). For emergence, metagenomic analyses were voted most often.
- The environmental compartment prioritized by most participants was surface water (133/198) with the objective to determine trends in the environment (64/198), followed by recreational water (38/198), in order to generate data contributing to risk assessment. No clear target emerged from the analysis – among 81 participants, 10 resp. 8 chose resistance genes by high-throughput PCR (HT-PCR) and qPCR, while 16 participants chose culturable bacteria (carbapenem or 3GC resistant Enterobacterales) – 17/81 participants stated that they would not know or would not have the capacity to decide.

5.2 Summary of final workshops on adoption of the common strategy

EU-WISH workshop Dublin

The EU-WISH workshop was held in Dublin, Ireland on 9-10 September 2025. Among other goals, the workshop aimed to agree on environmental common approaches and future strategies on surveillance for AMR.

EU-WISH had addressed AMR target prioritization through **mapping current AMR surveillance in member states** and by **conducting workshops within and beyond EU-WISH**. Based on these activities, the following preliminary general approach was suggested to the Dublin International Workshop participants:

- With the objective to determine temporal trends of AMR circulating in the human population, and to identify emergence of new forms of clinically-relevant AMR, surveillance should focus on **untreated wastewater influent**, with suggested harmonized surveillance targets being **quantitative analysis of carbapenem resistant Enterobacterales** (most importantly including *Escherichia coli* and *Klebsiella pneumonia*, by culture to assess trends) and **resistance genes in the wastewater metagenome to assess emergence of AMR**.

- With the objective to generate data on human exposure to AMR through surface water, and to study efficiency of wastewater treatment, surveillance is also recommended to include **treated wastewater effluent**, with a wider range of options for harmonized surveillance targets (to be further determined within EU-WISH and in collaboration with EU-JAMRAI2).

During the workshop, participants generally adopted this preliminary general approach for wastewater surveillance of AMR. Specific feedback and additions were provided for the following points:

- A group of experts favour testing of **a panel of resistance genes** to monitor temporal trends.

- **Characterization of bacterial isolates** by for example whole genome sequencing is considered to be important and will be further discussed within the working group.

- It is recommended that **temporal and regional adaptations** of the priority list are possible in addition to a minimum approach applied across European countries.

- Criteria for **selection of wastewater surveillance plants (WWTPs)** were discussed, such as a focus on specific WWTP for emergence of AMR at 'risk' sites, as well as procedures towards a common SOP. **Training needs** for AMR testing were also identified.

JAMRAI Workshop Madrid

A joint WP6.3/7.3/8.3 workshop was held in Madrid, Spain, from 13 to 15 October focusing on the presentation of latest results. Among other goals, the workshop aimed to agree on environmental common approaches and future strategies on surveillance for AMR.

A summary of the participants in the event is shown below in figures:

A first draft of the common approach was presented to the participants and discussed in small groups seeking their agreement and further comments. No group indicated disagreement. Most adjustments were textual or concerning clarification. Issues which needed to be addressed with more time were not of a fundamental nature.

5.3 Summary of prioritization in the survey

Survey participation

The survey was analysed by a 8.3 working group of 6 countries and 7 institutes. The results relevant for this milestone are presented here, all other results from the survey will be published through scientific publications and presentations.

- **Past & Present survey**: We received 42 responses, ranging from 1 to 5 per country. In total, surveillance systems were reported in 12 of the 16 participating countries (75%

coverage). A total of 53 surveillance systems were described, ranging from 1 to 19 systems per country.

- **Future survey**: Responses were treated as individual expert opinions. We received 42 responses.

Results

The survey results showed – as expected – that environmental AMR surveillance is a relatively recent field. Most surveillance systems were initiated between 2010 and 2015, with considerable expansion over the past five years—likely influenced by the rapid development of WBS during the COVID-19 pandemic.

1) Environmental compartments targeted and associated objectives

Current surveillance systems primarily focus on wastewater and surface water. Although soil was not frequently reported as a current compartment, it was considered nearly as important as wastewater and surface water for future surveillance efforts (Figure 1).

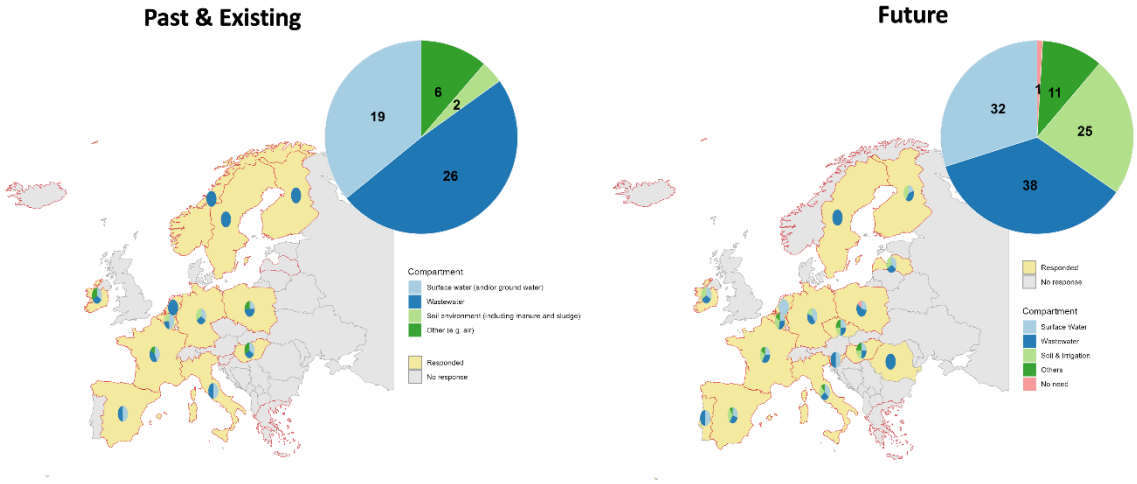


Figure 1: Overview of countries of origin of respondents and surveillance systems reported in both surveys on environmental surveillance.

2) Defining objectives for environmental AMR surveillance

Environmental AMR surveillance may serve multiple objectives. These must be clearly defined, as they shape the choice of sampling strategies, analytical methods, and AMR targets. Across both surveys, objectives were grouped into four categories. Since WBE, particularly its use to infer AMR in human populations, has been a major objective in past studies, it made a specific category in the "Past & Present".

The most reported and suggested objective, with a little more than a third of the responses, was "Provide information about patterns and trends in AMR". Second objectives suggested for future surveillance equally placed "Support and inform risk analysis of AMR in the environment", "Alert on emergence and evolution of AMR", and "Assess the effectiveness of interventions"

A cross-analysis of the Future survey responses showed that each objective is associated with different priority compartments:

To meet the objective "Provide information about patterns and trends in AMR", surveillance should primarily focus on wastewater and surface water.

For the objective "Assess the effectiveness of interventions", the recommended compartments are wastewater and soil or soil-related compartments.

3) AMR targets in current environmental AMR surveillance

To support the development of a harmonized EU-wide approach, the surveys assessed which targets and methodologies are currently used and which are recommended for the future. Since soil systems are likely under-represented in the current dataset, the analysis below focuses on wastewater and surface water.

Results pointed to a wide variety of AMR targets being used across countries. The most common are antibiotic resistance genes (ARGs). Indeed, ARGs were monitored in wastewater by all responding countries. In surface water, ARGs and antibiotic-resistant bacteria (ARBs) have been found in equal proportions.

- Most monitored gene families: Aminoglycosides, carbapenems/monobactams, tetracyclines, and trimethoprim/sulfamethoxazole were the most frequently monitored families of ARG in both surface water and wastewater. Untargeted approaches aiming to quantify all known ARGs were also widely used in wastewater.
- Most monitored resistant bacteria: The top monitored ARB were similar across compartments. *E. coli* / Enterobacterales, 3rd/4th-generation cephalosporin-resistant (3/4GCR) bacteria, and carbapenem-resistant bacteria were the most commonly targeted groups.

Proposed AMR targets for future surveillance

Both carbapenem/monobactam and 3rd/4th-generation cephalosporin resistance 3/4GCR emerge from the future survey as the most recommended ARG families and ARB to monitor. Interestingly, fluoroquinolones appear among the top five recommended ARG families, despite not being a major target in current surveillance systems. Similarly, carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were also identified as important target organisms for future monitoring.

Finally, as the survey did not force to prioritize one objective or AMR target above the other, the future survey might have provided a broader picture on possible objectives, compartments and targets than apparent in the prioritization exercises.

5.4 Considerations during the final synthesis of the common approach

During the formulation of the common approach, a number of decisions were taken, based on the following considerations:

The ultimate goal of environmental AMR surveillance is to reduce the impact of AMR on humans and animals.

The ultimate goal of surveillance of antimicrobial agents in this context is to determine their possible impact on AMR in the environment through selection, In addition, antimicrobial residues can affect environmental health (such as effects on diversity and functioning of environmental consortia). Both these surveillance goals were considered.

5.5 Summary of cross check with animal and human health professionals

During the online WP8 webinar of November 27 2025 several parts of the common approached were presented and questioned. The One Health consultation survey presented in this meeting will be analyzed later.

6 NEXT STEPS, WP8.3.2 THE PILOT

In 2026, a pilot will evaluate environmental surveillance according to the common approach. Preparation of the pilot has been initialized. At the Madrid meeting, a "Cookbook" has been worked on, and a more detailed definition of sample locations to be included in surface water sampling has been discussed.

7 REFERENCES

Bengtsson-Palme J, Abramova A, Berendonk TU, Coelho LP, Forslund SK, Gschwind R, Heikinheimo A, Jarquín-Díaz VH, Khan AA, Klümper U, Löber U. (2023) Towards monitoring of antimicrobial resistance in the environment: For what reasons, how to implement it, and what are the data needs?. Environment International. 178:108089.

Iera J, Isonne C, Seghieri C, Tavoschi L, Ceparano M, Sciurti A, D'Alisera A, Sane Schepisi M, Migliara G, Marzuillo C, Villari P. (2025) Availability and key characteristics of National Early Warning Systems for emerging profiles of antimicrobial resistance in high-income countries: systematic review. JMIR Public Health and Surveillance. 11:e57457.

Larsson DJ, Flach CF. (2022) Antibiotic resistance in the environment. Nature Reviews Microbiology. 20(5):257-69.

Liguori K, Keenum I, Davis BC, Calarco J, Milligan E, Harwood VJ, Pruden A. (2022) Antimicrobial resistance monitoring of water environments: a framework for standardized methods and quality

control. Environmental science & technology. 56(13):9149-60. https://pmc.ncbi.nlm.nih.gov/articles/PMC9261269/

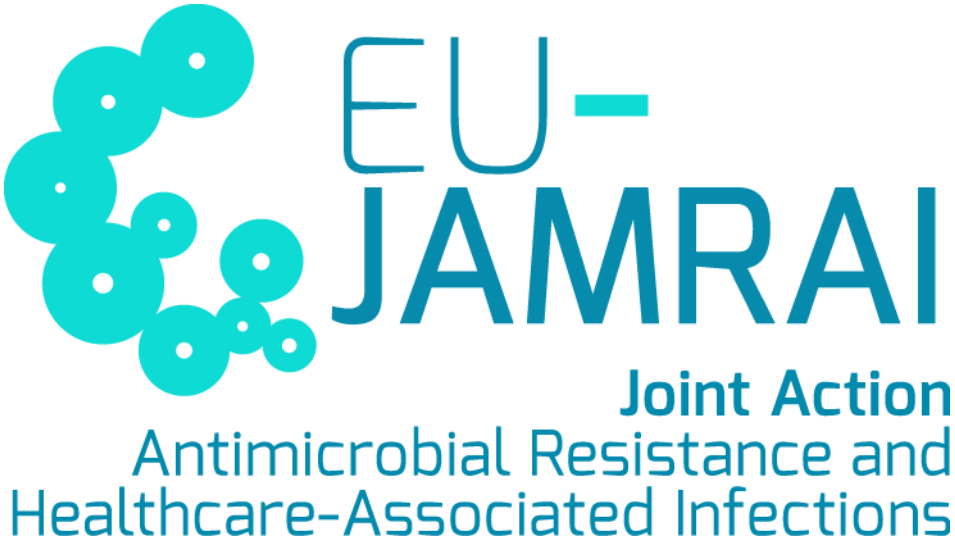
United Nations General Assembly (2024). Political Declaration of the High-Level Meeting on Antimicrobial Resistance : resolution / adopted by the General Assembly

EU Council (2023) Council recommendation on stepping up EU actions to combat antimicrobial resistance in a One Health approach (2023/C 220/01)

Schwermer C et al. (2025) Pilot study on antimicrobial resistance monitoring in European surface waters – Final report of the Eionet Working Group

WHO (2024) Wastewater and environmental surveillance for one or more pathogens, Guidance on prioritization, implementation and integration (Draft report). https://www.who.int/publications/m/item/wastewater-and-environmental-surveillance-for-one-or-more-pathogens--guidance-on-prioritization--implementation-and-integration

United Nations Environment Programme - UNEP (2023) Bracing for Superbugs : Strengthening environmental action in the One Health response to antimicrobial resistance



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