EU-JAMRAI-2 Survey on future environmental AMR surveillance in Europe - goals, sampling, indicators and challenges

Fields marked with * are mandatory.

1 Introduction

Background

Antimicrobial resistance (AMR) is one of the most serious cross-border threats to health. To mitigate AMR, a One Health approach is needed, considering the interconnection between humans, animals and the environment.

Following the 2017 EU One-Health Action Plan against AMR and the 2023 Council Recommendation, the European Commission has financed a 2nd European Joint Action on AMR and Healthcare-associated infections (EU-JAMRAI-2) within the EU4Health programme. This project brings together 128 partners from the EU, Iceland, Norway and Ukraine, and will last from 2024 to 2027. For more information on EU-JAMRAI-2, please visit the website here: https://eu-jamrai.eu/.

EARS-Env
EU-JAMRAI-2 aims to establish a European One Health community among countries, institutions, and sectors to combat AMR. Among its activities is the development of a European surveillance network for AMR in the environment: EARS-Env. Within EARS-Env, the objectives and setup of environmental AMR surveillance (matrices, samples, and indicators) will be summarized, and a blueprint and guidance for a common environmental AMR surveillance will be developed, and subsequently piloted in the 16 participating countries. “The environment” is understood as the living environment (water, soil, air), including modifications by various discharges (wastewater, hospital effluents), or amendments (organic amendment, slurry, manure, sludge).

Surveys
To achieve this, two separate but related surveys have been developed. The outcomes of the surveys will serve as input for the development of the above-mentioned guidance and protocols for environmental AMR surveillance. The surveys address two objectives:

A) the first survey aims to draw up an inventory of existing AMR monitoring of environmental compartments (wastewaters, soils etc) (please use this link: Survey A: Existing environmental surveillance).
B) this survey will examine surveillance objectives, samples / matrices and indicators desirable for future environmental AMR surveillance.

Survey B Structure: Future Environmental Surveillance

The survey has the following structure:

- Consent
- Participant profile
- Future environmental surveillance
- Satisfaction
- Annexes

Survey B on future environmental surveillance includes general and specific objectives, urgent and long-term signals, sampling strategies, indicators and methods for future environmental surveillance according to your opinion.

Deadlines

Please fill in ASAP

Annexes (top right corner of the screen)

A list of human and veterinary antimicrobials and AMR genes is available in Annex 1.
A list of term definitions together with the related EU regulations is available in Annex 2.

Contact

For questions, please email your national contact point.

Thank you very much for your valuable contributions.

On behalf of the full team of EU-JAMRAI 2 work package 8.3 - Roosmarijn Luiken, Luis Lucena, Thibault Stalder,

Christophe Dagot and Heike Schmitt.

2 Practical instructions and FAQ's

IMPORTANT WHEN SUBMITING YOUR RESPONSE:

Due to technical issues, there is a need to wait around 10 minutes for the survey's submission button to appear when finalising your response. After pressing the submission button, one should land in a confirmation page where you can download your pdf submission containing a contribution ID to your indicated email.

Please remember to save a draft when completing the survey and right before submitting. When reloading your draft response from your draf link, it may appear blank but it can take around 10 minutes for all the saved answers to appear, so you need to wait until then to submit. If you submitted your response and you did not land in a confirmation page, you most probably did not succeed in the submitting process.

It is also not possible to add more than 3 surveillance systems per survey as there will be difficulties when submitting. Therefore, we recommend you create another survey response for additional surveillance systems.

For any issue encountered after following our recommendations, please contact directly the email indicated in our EU survey at the right panel.

What is understood as surveillance in this survey?

For this questionnaire, a surveillance system is defined as a structured approach targeting specific environmental compartments (such as wastewater, or surface water, or soil), with a set selection of sampling sites, frequencies of sampling and indicators such as specific resistant bacteria and/or resistance genes.

Who can fill in the questionnaire?

This questionnaire is open to experts involved in current environmental surveillance activities, as well as in surveillance in other domains (human or animal surveillance). Experts from different backgrounds can participate, such as governmental and academic scientists. Also, governmental representatives, members of NGOs and waterboards can fill in this questionnaire.

Answers can be submitted directly. However, for some countries, answers are collected by one national contact point. If you are aware of other experts who should be consulted, please let the contact person in your country know.

Survey platform

The questionnaire is publicly available and runs on the EU survey platform. It can be answered without an EU login. Please be aware that we will not accept any response or data outside this platform.

How long will it take me to fill in this survey?

The estimated time to complete this survey ranges from 1 to 4 hours, depending mainly on the number of matrices to be reported.

Can I save a draft while working on the survey?

Respondents can save drafts multiple times and are encouraged to test this functionality early to avoid data loss. Ensure your final draft is saved before submission.

Can my contribution be modified or submitted after the submission deadline?

Only in exceptional circumstances (e.g. technical issues or data errors) will it be possible to extend the deadline or allow re-submission. We strongly recommend that you allow yourself sufficient time ahead of the submission deadline to input your response. We also recommend you save your draft frequently and review thoroughly before final submission.

How will the results be used?

Survey responses will guide the development of standardized protocols and sampling strategies for environmental surveillance, contributing to more integrated systems aligned with the One Health framework.

After data analysis, interpretation and consultation, the results of all contributing responses in this survey will be published in a scientific journal.

A pilot on environmental AMR surveillance will be organized in a second phase of EU-JAMRAI-2.

Will my contribution to this survey be anonymous?

Yes, the name of each specific respondent and all accompanying personal data (email addresses, etc.) will be strictly anonymized in the resulting scientific publication, deliverables, EU-JAMRAI-2 policy reports and in any other communication and dissemination materials.

Will my contribution to this survey be acknowledged?

All contributors will be acknowledged in publications in a special acknowledgement section in the form they prefer (ie, name and/or institution). If you are interested in a more formal recognition of your contribution (e. g. co-authorship with associated responsibilities) you can let us know via your country contact point.

How will my personal data be used?

As this online service collects and further processes personal data, Regulation (EU) N° 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data, is applicable.

The personal data collected and further processed are data necessary for the participation in this questionnaire, namely organization, your view on the topics subject to the survey, country of residence and your contact details (name and email of the contributors). These will be only used to contact the respondents in case we have additional questions (e.g. whether SOPs could be supplied).

For the collection of data in this survey, we rely on the EU Survey external system. For more information on how EU Survey processes personal data, please see: https://ec.europa.eu/eusurvey/home /privacystatement.

How long do we keep your data?

Your personal data will remain in the database until the results have been completely analyzed and exploited for EU-JAMRAI-2. The project EU-JAMRAI-2 ends in December 2027.

3 Consent

Your consent to the processing of your data

When you submit this questionnaire, you consent that EU-JAMRAI-2 will process your personal data provided in the questionnaire as explained in this data protection statement. You may also withdraw your consent later at any time. However, this will not affect the lawfulness of any data processing carried out before your consent is withdrawn.

* 3.1 Please confirm that you have read and understood the Data Protection Statement above and that you consent to the processing of your personal data:
  - ○ Yes
  - ○ No

* 3.2 Please confirm that you consent to the publication of your anonymized survey responses in EU-JAMRAI-2 reporting and possible scientific publications:
  - ○ Yes
  - ○ No

* 3.3 Please confirm that you consent to possibly be contacted by EU-JAMRAI-2 survey organizers in relation to your responses to support the finalization of this survey:
  - ○ Yes
  - ○ No

* 3.4 I would like to be acknowledged in the acknowledgement section of a possible publication:
  - ○ Yes
  - ○ No

* 3.5 For acknowledgements, please use the following (we suggest name and institution but are open to other possibilities):

50 character(s) maximum

4 Participant profile

* 4.1 Which country are you working in?
  - ○ Afghanistan
  - ○ Albania
  - ○ Algeria

- American_Samoa
- Andorra
- Angola
- Anguilla
- Antigua_and_Barbuda
- Argentina
- Armenia
- Aruba
- Australia
- Austria
- Azerbaijan
- Bahamas
- Bahrain
- Bangladesh
- Barbados
- Belarus
- Belgium
- Belize
- Benin
- Bermuda
- Bhutan
- Bolivia
- Bosnia_and_Herzegovina
- Botswana
- Brazil
- British_Virgin_Islands
- Brunei_Darussalam
- Bulgaria
- Burkina_Faso
- Burundi
- Cambodia
- Cameroon
- Canada
- Cape_Verde
- Cayman_Islands
- Central_African_Republic
- Chad
- Chile
- China
- Colombia
- Comoros
- Congo
- Costa_Rica
- Cote_dIvoire
- Croatia
- Cuba
- Curaçao

- Cyprus
- Czechia
- Dem_Peoples_Rep_of_Korea
- Democratic_Republic_of_the_Congo
- Denmark
- Djibouti
- Dominica
- Dominican_Republic
- Ecuador
- Egypt
- El_Salvador
- Equatorial_Guinea
- Eritrea
- Estonia
- Eswatini
- Ethiopia
- Faroe_Islands
- Fiji
- Finland
- France
- French_Polynesia
- Gabon
- Gambia
- Georgia
- Germany
- Ghana
- Gibraltar
- Greece
- Greenland
- Grenada
- Guam
- Guatemala
- Guernsey
- Guinea
- Guinea_Bissau
- Guyana
- Haiti
- Holy_See
- Honduras
- Hungary
- Iceland
- India
- Indonesia
- Iran
- Iraq
- Ireland
- Isle_of_Man

- Israel
- Italy
- Jamaica
- Japan
- Jersey
- Jordan
- Kazakhstan
- Kenya
- Kosovo
- Kuwait
- Kyrgyzstan
- Laos
- Latvia
- Lebanon
- Lesotho
- Liberia
- Libya
- Liechtenstein
- Lithuania
- Luxembourg
- Madagascar
- Malawi
- Malaysia
- Maldives
- Mali
- Malta
- Marshall_Islands
- Mauritania
- Mauritius
- Mexico
- Moldova
- Monaco
- Mongolia
- Montenegro
- Montserrat
- Morocco
- Mozambique
- Myanmar
- Namibia
- Nepal
- Netherlands
- New_Caledonia
- New_Zealand
- Nicaragua
- Niger
- Nigeria
- North_Macedonia

- Northern_Mariana_Islands
- Norway
- Oman
- Pakistan
- Palau
- Palestine
- Panama
- Papua_New_Guinea
- Paraguay
- Peru
- Philippines
- Poland
- Portugal
- Puerto_Rico
- Qatar
- Romania
- Russia
- Rwanda
- Saba
- Saint_Kitts_and_Nevis
- Saint_Lucia
- Saint_Vincent_and_the_Grenadines
- Samoa
- San_Marino
- Sao_Tome_and_Principe
- Saudi_Arabia
- Senegal
- Serbia
- Seychelles
- Sierra_Leone
- Singapore
- Sint_Eustatius
- Sint_Maarten
- Slovakia
- Slovenia
- Solomon_Islands
- Somalia
- South_Africa
- South_Korea
- South_Sudan
- Spain
- Sri_Lanka
- Sudan
- Suriname
- Sweden
- Switzerland
- Syria

- Taiwan
- Tajikistan
- Thailand
- Timor_Leste
- Togo
- Tonga
- Trinidad_and_Tobago
- Tunisia
- Turkey
- Turks_and_Caicos_islands
- Uganda
- Ukraine
- United Kingdom
- United_Arab_Emirates
- United_Kingdom
- United_Republic_of_Tanzania
- United_States_of_America
- United_States_Virgin_Islands
- Uruguay
- Uzbekistan
- Vanuatu
- Venezuela
- Vietnam
- Wallis_and_Futuna
- Western_Sahara
- Yemen
- Zambia
- Zimbabwe

* 4.2 What type of institution do you work for?

between 1 and 13 choices

- [ ] Ministry of Environment
- [ ] Ministry of Health
- [ ] Ministry of Agriculture
- [ ] Ministry, other
- [ ] Governmental institute (environmental domain / environmental protection agency)
- [ ] Governmental institute (human / public health domain)
- [ ] Governmental institute (animal health domain)
- [ ] Governmental institute (other)
- [ ] Research Institution / Academia / University
- [ ] Healthcare institution
- [ ] NGO / non-profit organisation
- [ ] Waterboard / water sector
- [ ] Other

4.3 Other institution:

100 character(s) maximum

* 4.4 At which territorial scale do you mainly work in your country?

- ○ Supranational
- ○ National
- ○ Regional
- ○ Local
- ○ Other

4.5 Please describe 'other'

50 character(s) maximum

4.6 Can you give contact details of the person / persons that helped complete this survey, one for each surveillance system that is included in your answers? If it is just you/one person, just fill one row.

	Name of institution	Type of institution	Name and surname of contact person	Email	Name of surveillance system for which the contact person provided details
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

5 Future surveillance: Objectives, sampling strategy and methods for future environmental surveillance

* 5.1 Do you believe there is a need for a future (next 5 to 10 years) surveillance system for antimicrobial resistance or related pollutants (e.g. antibiotics or fungicides) in any of the following environmental compartments? *(click all that apply)*

- ☐ yes, in wastewater
- ☐ yes, in inland water (including surface/ground water)
- ☐ yes, in soil and/or related environments, biosolids and irrigation water
- ☐ yes, in another environmental compartment (e.g. transport locations, air, etc.)
- ☐ no, there is no need for environmental surveillance

5.2 Please specify which 'other' environmental compartment:

50 character(s) maximum

5.3 Do you want to explain?

500 character(s) maximum

5.1 Environmental compartment

5.1.1 If yes, for which of these environmental compartments do you want to describe your vision/expert opinion on the design of the future surveillance? *(we advise maximally 2 depending on your expertise)*

- ☐ yes, in wastewater
- ☐ yes, in surface and/or ground water
- ☐ yes, in soil and/or biosolids/irrigation water
- ☐ yes, in another environmental compartment (e.g. transport locations, air, etc.)

The following questions are related to your vision/expert opinion for the future regarding environmental surveillance for the compartment of your choice. They contain questions on the objectives of environmental surveillance, starting from general purposes which are then broken down into more specific purposes. Followed by questions on sampling, targets, lab methods, finances and more.

Future: next 5 years

The questions address a time-frame of the next 5 years. Therefore we ask you to consider surveillance systems you believe will be possible to implement within the next 5 years, even if the required methodology is not yet available in your country and/or the exact outline still needs to be established.

Important: Many questions are mandatory, however they all have the option 'I don't know'. Please use this option when applicable.

5.2 Future surveillance - Wastewater-based surveillance

In this section, when we speak about 'environment' or 'environmental surveillance' we mean WASTEWATER surveillance. Please keep that in mind when answering.

5.2.1 Future surveillance - Objectives of surveillance

5.2.1.1 **General objectives**

Which of the following general objectives should be addressed by AMR surveillance in the environment? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Provide information about patterns and trends in AMR							
* Support and inform risk assessment of AMR in the environment (including informing exposure assessments)							
* Alert on emergence and evolution of AMR							
* Assess the effectiveness of interventions							

5.2.1.2 **Specific objectives– Patterns and trends in AMR**

If environmental AMR surveillance should provide information about patterns and trends in AMR, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Determine spatial variations in the levels of AB /ARB/ARGs within the area under surveillance							
* Determine temporal variations in the levels of AB/ARB/ARGs within the area under surveillance							
* Identify and monitor transmission of clinically relevant AMR mechanisms across geographical borders							
*							

Identify and monitor sources of emissions of AMR (from humans and/or animals) as they contaminate the environment							

5.2.1.3 **Specific objectives– data for risk assessments**

If environmental AMR surveillance should deliver data to inform risk assessments, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to human health (e.g. data enabling determination of human exposure to AMR in the environment)							
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to animal health							
* Use environmental surveillance to generate data helping to assess the risks of AMR, including antibiotics and related pollutants, to environmental health (such as to aquatic life)							
* Identify and monitor transmission of clinically relevant AMR mechanisms from humans to animals and from animals to humans							
* Apply environmental surveillance for tracing of outbreaks							
* Apply environmental surveillance to assess environmental law offences							

5.2.1.4 Other ?

100 character(s) maximum

5.2.1.5 **Specific objectives - Emergence and evolution of AMR**

If emerging forms of AMR should be monitored within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5		I don't know/I prefer

						Not important at all	not to answer
* Generate data on the emergence of new clinically relevant resistance mechanisms							
* Identify and assess hotspots for emergence, genetic transmission and evolution of AMR							
* Identify and monitor predictors and drivers of AMR diversity and abundance							
* Identify and monitor transmission of clinically relevant AMR mechanisms across bacterial species							

5.2.1.6 Other ?

100 character(s) maximum

5.2.1.7 **Specific objectives – Efficiency of interventions**

If determining the efficiency of interventions to curb AMR should be pursued within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Evaluate treatment methods for AMR removal at centralised wastewater utilities							
* Evaluate the efficiency of changes in antibiotic stewardship and/or infection prevention and control within human or animal populations							
* Evaluate treatment methods for AMR removal at hospital wastewater facilities							
* Evaluate treatment methods for AMR removal during sludge treatment							

5.2.1.8 Other ?

100 character(s) maximum

Signals for urgently actionable public health interventions

In the field of public health surveillance of communicable diseases, some health threats require urgent action among competent national authorities in order to initiate a response (e.g. COVID-19). With a view to ascertain which AMR signals should require such prompt response, if any, a series of scenarios have been listed in the question below.

5.2.1.9 Which of the following AMR signals in the environment should trigger URGENTLY ACTIONABLE INTERVENTIONS? (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Increase in the levels of clinically relevant ARB/ARGs	○	○	○	○	○	○	○
* Increase in the levels of any antibiotic	○	○	○	○	○	○	○
* Increase in the levels of antibiotic residues	○	○	○	○	○	○	○
* Transmission of clinically relevant AMR mechanisms in water environments across geographical borders	○	○	○	○	○	○	○
* Persistence in time of AMR determinants	○	○	○	○	○	○	○
* Emergence of new clinically relevant resistance mechanisms	○	○	○	○	○	○	○
* Increase of AMR drivers (antibiotics, biocides, heavy metals, microplastics, etc)	○	○	○	○	○	○	○
* Increase in biomarker indicators of transmissibility of ARGs (ex. mobile genetic elements)	○	○	○	○	○	○	○
* Transmission of clinically relevant AMR mechanisms in water environments across human-animal species	○	○	○	○	○	○	○
* Transmission of clinically relevant AMR mechanisms in water environments across bacterial species	○	○	○	○	○	○	○
* Increase in the levels of AMR-related toxicity (e.g. water biodiversity loss)	○	○	○	○	○	○	○

5.2.1.10 Other ?

100 character(s) maximum

Exploring signals for public health surveillance in wastewater

As outlined in the new (recast) Urban Wastewater Treatment Directive 2024, in case of water pollution (e.g. discharges of UWW) affecting neighbouring member states, transboundary cooperation is necessary in order to prompt a swift notification to competent authorities for a timely response.

5.2.1.11 In future, do you agree with the idea of needing to develop a cross-border one-health early warning and response alert system of AMR in wastewater? (Respond 2 if you don't have an opinion)

5.2.1.12 Do you agree with the idea that AMR detection in wastewater could be a reflection of the resistant microbial communities and biofilms existing within the piping system instead of the circulation of AMR in the human population?

Exploring the role of hospital wastewater for monitoring of AMR

5.2.1.13 Are you willing to answer 8 questions about AMR surveillance in hospital wastewater?

- Yes
- No

5.2.1.14 To which extent do you agree with the idea that AMR detection in hospital wastewater could improve the management of hospital outbreak detection and investigation?

5.2.1.15

To which extent do you agree with the idea that AMR and/or antibiotics detection in hospital wastewater could improve adjusting antibiotic prescription stewardship (e.g., management of resistance to last-resort antibiotics) at the hospital level based on existing AMR indicators?

5.2.1.16 To which extent do you agree with the idea that AMR detection in hospital wastewater could improve the management of hospital effluents based on existing AMR indicators in the local water environment?

According to WHO, Infection Prevention and Control (IPC) interventions in healthcare facilities, such as hand hygiene, and ensuring access to high-quality water, sanitation and hygiene (WASH) services can reduce the risk of health care-associated infections (HAIs) by up to 70% and have a high economic return on investment. Recently, WHO has published a global framework for action and monitoring for 2024–2030 for IPC and WASH.

5.2.1.17

To which extent do you agree with the idea that AMR detection in hospital wastewater could improve the evaluation of the efficiency of WASH and IPC practices?

5.2.1.18
In future, to which extent do you agree with the idea of needing to develop a mandatory routine monitoring of hospital wastewater as a national surveillance system of AMR in hospital environments?

* 5.2.1.19
In future, which of the following operators should be held responsible for carrying out such AMR surveillance in hospital wastewaters?

- [ ] Hospital professionals
- [ ] Public health institutions
- [ ] Economic operators of the water sector
- [ ] Independent government agencies/associations
- [ ] Independently contracted agencies
- [ ] Universities
- [ ] Other
- [ ] I prefer not to answer/I don't know

5.2.1.20 Please specify 'other':
50 character(s) maximum

5.2.1.21 The AMR Industry Alliance has developed a third-party certification scheme for antibiotic manufacturers to promote and demonstrate responsible antibiotic manufacturing in the global pharmaceutical supply chain. To which extent do you agree with the idea of needing to develop such a third-party certification scheme for demonstrating good hospital wastewater management practices?

* 5.2.1.22 If so, which of the following hospital environments would you prioritise as sampling sites?

- [ ] Hospital wastewater
- [ ] Hospital water pipes
- [ ] Hospital sinks and drains
- [ ] Housekeeping equipment
- [ ] High touch surfaces
- [ ] ICU areas
- [ ] Other
- [ ] I prefer not to answer/I don't know

5.2.1.23 Please specify 'other':
100 character(s) maximum

5.2.1.24 Would there be fundamental changes to your answers on 'objectives' if you were to consider the far future (10 years) instead of the next 5 years? if so, please explain:

300 character(s) maximum

5.2.2 Future surveillance - Sampling

Sites

5.2.2.1 **Wastewater-based surveillance**

Wastewater-based surveillance is understood here as surveillance directed at determining the circulation of AMR determinants in the human and animal population, but not in the wider environment (the determination of AMR inn effluents in order to characterise emissions to the environment is included).

In future, which of the following environmental sub compartment is essential to monitor for wastewater-based surveillance?

between 1 and 2 choices

- [ ] Animal husbandry waste water
- [ ] Aquaculture waste water
- [ ] Hospital wastewater
- [ ] Industrial effluents
- [ ] Inlet and outlet of centralised, municipal wastewater treatment plants
- [ ] Inlet of centralised, municipal wastewater treatment plants
- [ ] Other
- [ ] Outlet of centralised, municipal wastewater treatment plants
- [ ] Reused water
- [ ] Urban water runoff
- [ ] Wastewater from healthcare clinics
- [ ] Wastewater from long-term care facilities, nurseries

5.2.2.2 Please specify 'other':

100 character(s) maximum

Frequency

5.2.2.3 In future, how frequently should the surveillance be undertaken?

- ( ) Once annually
- ( ) Twice a year
- ( ) Three times a year
- ( ) Every three months
- ( ) Every month
- ( ) Daily

- ◯ Real time

* 5.2.2.4 In future, to which of the following NATURAL phenomena should environmental AMR surveillance adapt in order to be more representative? (1-3 answers if needed)

Maximum 3 selection(s)

- ☐ Distribution across all seasons
- ☐ Cold seasons
- ☐ Hot seasons
- ☐ The first rainfall of a season
- ☐ Events of heavy rainfall and storm overflow
- ☐ Drought period
- ☐ Sand and dust storms
- ☐ Extreme weather disasters (e.g earthquakes, volcanic eruptions, hurricanes, tsunamis)
- ☐ Bird/fish migration season
- ☐ Pollination season
- ☐ None of the above
- ☐ Other
- ☐ I don't know / prefer not to answer

5.2.2.5 Please specify 'other':

100 character(s) maximum

* 5.2.2.6 In future, to which of the following periods impacted by human activities should environmental AMR surveillance in wastewater adapt to in order to be most representative? (One answer - 3 max if necessary)

Maximum 3 selection(s)

- ☐ Pesticide application periods
- ☐ Food harvest periods
- ☐ Events of treated industrial emissions
- ☐ Periods of increased incidence in human infections (e.g. respiratory disease period in winter, gastroenteritis in summer)
- ☐ Periods of increased incidence in infectious outbreaks across livestock and aquaculture
- ☐ Periods of increased incidence in pests in agriculture
- ☐ Periods of touristic travelling
- ☐ Bathing season
- ☐ Mass gatherings: religious pilgrimages (Hajj), music, sport and festivity events (Olympics, Oktoberfest)
- ☐ None of the above
- ☐ Other
- ☐ I don't know / prefer not to answer

5.2.2.7 Please specify 'other':

100 character(s) maximum

The role of AMR in global health and climate change

5.2.2.8 In your opinion, will climate change influence AMR in such a way that this should be taken into consideration for AMR surveillance in wastewater, if so, please indicate how.

500 character(s) maximum

* 5.2.2.9 In your opinion, which of the following future scenarios impacted by human activities should AMR surveillance in wastewater adapt to in order to be more representative?

- [ ] CBRN incidents (chemical, biological, radiological and nuclear)
- [ ] Periods of armed conflicts, war and mass casualties
- [ ] Mass migration across geographical borders
- [ ] Direct non-treated industrial discharges into the environment (e.g. shipwrecks emitting industrial substances into marine waters, leakages from pharmaceutical or hospital facilities into rivers, etc)
- [ ] Outbreaks derived from high-containment laboratories for high threat pathogens (e.g. biosafety level-4 laboratories, gain-of-function research labs)
- [ ] Severe foodborne illness/outbreaks events (e.g. *E. coli* O104:H4 outbreak)
- [ ] During any ongoing public health emergency of international concern
- [ ] None of the above
- [ ] I don't know /I prefer not to answer
- [ ] Other

5.2.2.10 Please specify 'other':

50 character(s) maximum

* 5.2.2.11 The new (recast) EU Urban Wastewater Treatment Directive calls for identification of vulnerable populations. In future, which of the following (vulnerable) populations should and practically can be the focus of the AMR surveillance in wastewater?

- [ ] Victims of CBRN incidents (chemical, biological, radiological and nuclear)
- [ ] War victims, displaced people, people in refugee camps
- [ ] Elderly
- [ ] Students
- [ ] Chronically ill and disabled
- [ ] Low-income, homeless individuals and children in foster care
- [ ] Rural isolated populations
- [ ] Racial or ethnic minorities (e.g. indigenous or immigrant communities)
- [ ] Institutionalized persons (for example, persons in correctional facilities, nursing homes or mental health facilities)
- [ ] People engaging in criminal activities (e.g. use of illegal substances)
- [ ] Sex workers and victims of sexual trafficking
- [ ] Employees of the industry sector (e.g. wastewater, landfill)
- [ ] Farmers
- [ ] Health professionals (e.g. medical doctors, veterinarians, nurses)
- [ ] People residing in areas with non-sewered sanitation
- [ ] People residing in areas near WWTPs
- [ ]

None of the above

- [ ] Other
- [ ] I don't know /I prefer not to answer
- [ ] Children and pregnant women

5.2.2.12 Please specify 'other':

150 character(s) maximum

5.2.2.13 Would there be fundamental changes to your answers on 'sampling', ie sites and frequency, if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.2.3 Future surveillance - AMR indicators

* 5.2.3.1 **Identifying and monitoring antibiotic resistant bacteria (ARB)**

Which of the following bacterial targets would be the MOST FEASIBLE AND INFORMATIVE option for culture-based environmental surveillance of AMR?

- [ ] Aeromonas spp
- [ ] *Acinetobacter baumanii*
- [ ] *Bordetella pertussis*
- [ ] Campylobacter spp
- [ ] *Citrobacter freundii*
- [ ] *Corynebacterium diphteriae*
- [ ] *Clostridium perfringens*
- [ ] *Clostridioides difficile*
- [ ] Enterococcus spp
- [ ] *Escherichia coli*
- [ ] Shiga toxin/verocytotoxin-producing *Escherichia coli*
- [ ] *Haemophilus influenzae*
- [ ] *Klebsiella pneumonia*
- [ ] *Listeria monocytogenes*
- [ ] Legionella spp
- [ ] *Neisseria gonorrhoeae*
- [ ] *Neisseria meningitidis*
- [ ] *Pseudomonas aeruginosa*
- [ ] Salmonella spp
- [ ] Shigella spp
- [ ] *Staphylococcus aureus*
- [ ] *Streptococcus pneumoniae*
- [ ] *Vibrio cholera*
- [ ] Total coliform bacteria
- [ ] Others

- [ ] I don't know/I prefer not to answer

5.2.3.2 Please specify 'other':

100 character(s) maximum

* 5.2.3.3 Which of the following antibiotic-resistant groups of bacterial human pathogens from the WHO Bacterial Priority Pathogens list 2024 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR?

Maximum 15 selection(s)

- [ ] *Acinetobacter baumannii* carbapenem-resistant
- [ ] Enterobacterales third-generation cephalosporin-resistant
- [ ] Enterobacterales carbapenem-resistant
- [ ] *Enterococcus faecium* vancomycin-resistant
- [ ] *Haemophilus influenzae* ampicillin-resistant
- [ ] *Mycobacterium tuberculosis* rifampicin-resistant
- [ ] *Neisseria gonorrhoeae* third-generation cephalosporin, and/or fluoroquinolone-resistant
- [ ] *Pseudomonas aeruginosa* carbapenem-resistant
- [ ] *Salmonella Typhi* fluoroquinolone-resistant
- [ ] Non-typhoidal Salmonella fluoroquinolone-resistant
- [ ] Shigella spp. fluoroquinolone-resistant
- [ ] *Staphylococcus aureus* methicillin-resistant Group A
- [ ] Streptococci macrolide-resistant
- [ ] *Streptococcus pneumoniae* macrolide-resistant Group B
- [ ] Streptococci penicillin-resistant
- [ ] Others
- [ ] I don't know / I prefer not to answer

5.2.3.4 Please specify 'other':

100 character(s) maximum

* 5.2.3.5 Which of the following fungal human pathogens from the WHO fungal priority pathogens list 2022 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR surveillance?

Maximum 15 selection(s)

- [ ] *Aspergillus fumigatus*
- [ ] *Candida albicans*
- [ ] *Candida auris*
- [ ] *Candida parapsilosis*
- [ ] *Candida tropicalis*
- [ ] *Coccidioides spp*
- [ ] *Cryptococcus gattii*
- [ ] *Cryptococcus neoformans*
- [ ] *Eumycetoma* causative agents
- [ ] Fusarium spp

- Histoplasma spp
- *Lomentospora prolificans*
- Mucorales
- *Nakaseomyces glabrata (Candida glabrata)*
- Paracoccidioides spp
- *Pichia kudriavzeveii (Candida krusei)*
- Pneumocystis jirovecii
- Scedosporium spp
- *Talaromyces marneffei*
- Others
- I don't know /I prefer not to answer

5.2.3.6 Other:

100 character(s) maximum

Identifying and monitoring antibiotic resistant genes (ARGs)

According to NCBI's bacterial genomic data, the highest diversity of submitted AMR gene sequences corresponds to resistance to beta-lactams, followed by aminoglycosides, quinolones, glycopeptides, tetracyclines and macrolides, among others.

* 5.2.3.7 Which of the following antibiotic resistance classes should be prioritized for the purpose of AMR surveillance in wastewater?

Maximum 15 selection(s)

- Aminoglycoside resistance
- Amphenicol resistance
- Carbapenem and monobactam resistance
- Cephalosporins (First- and second-generation) resistance
- Cephalosporins (Third- and/or fourth-generation) resistance
- Fluoroquinolone resistance
- Glycopeptide resistance
- Imidazole derivative resistance
- Lincosamide and Streptogramin resistance
- Macrolide resistance
- Penicillin resistance
- Polymyxin resistance
- Tetracycline resistance
- Trimethoprim/sulphonamide resistance
- Other
- I don't know /I prefer not to answer

5.2.3.8 Other:

100 character(s) maximum

5.2.3.9 For each of the indicator purposes shown in the table below, which antimicrobial resistance gene targets would be the best for qPCR-based AMR surveillance in wastewater? Choose up to 10 genes for each category. You can use the genes shown in table 3 in the annex 1 or add other genes you might know.

	AMR proxy / anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

5.2.3.10 How would you allocate the following types of gene indicators across environmental compartments for AMR surveillance and risk assessment purposes?

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Surface water	☐	☐	☐	☐	☐
Ground water	☐	☐	☐	☐	☐
Wastewater	☐	☐	☐	☐	☐
Water reuse	☐	☐	☐	☐	☐
Sludge (WWTP)	☐	☐	☐	☐	☐
Manure	☐	☐	☐	☐	☐
Soil	☐	☐	☐	☐	☐

5.2.3.11 How would you allocate the following types of gene indicators by the following objectives of AMR surveillance in wastewater?

The above used objectives were:
1) Provide information about patterns and trends in AMR (including identification of emission sources)
2) Support and inform risk analysis of AMR in the environment
3) Alert on emergence and evolution of AMR
4) Assess the effectiveness of interventions

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Monitor trends of AMR	☐	☐	☐	☐	☐
Provide data for risk assessment	☐	☐	☐	☐	☐
Determine emerging AMR forms	☐	☐	☐	☐	☐
Evaluate interventions	☐	☐	☐	☐	☐

5.2.3.12 ***Identifying and monitoring levels of antibiotic and antibiotic residues***

Which of the following antimicrobial classes should be monitored for AMR risk assessment in wastewater?

Antibiotics	Antibiotics *Maximum 15 selection(s)* ☐ Aminoglycosides ☐ Amphenicols ☐ Carbapenems and monobactams ☐ First- and second-generation cephalosporins ☐ Fluoroquinolones ☐ Glycopeptides ☐ Imidazole derivatives ☐ Lincosamides and Streptogramins ☐ Macrolides ☐ Other ☐ Penicillins ☐ Polymyxins ☐ Tetracyclines ☐ Third- and/or fourth-generation cephalosporins ☐ Trimethoprim/sulphonamides
Antimycotics	Antimycotics *Maximum 5 selection(s)* ☐ Azoles ☐ Amphotericin B ☐ Echinocandins ☐ Terbinafine ☐ Others
Disinfectants / preservatives	Disinfectants / preservatives ☐ Triclosan ☐ Quaternary ammonium compounds QACs ☐ Chlorhexidine ☐ Chlorine-releasing compounds ☐ Aldehyde-based compounds ☐ Alcohols ☐ Hydrogen peroxide ☐ Peracetic acid ☐ Weak organic acids ☐ Others

* 5.2.3.13 **Defining criteria for risk assessment of antibiotics and antibiotic residues**

Which of the following already established indicators should be used to assess the selective potency of antibiotics for environmental risk assessments?

- ☐ Lowest Observed Effect Concentration (LOEC)
- ☐ No Observed Effect Concentration (NOEC)
- ☐ Predicted No Effect Concentration (PNEC)
- ☐ Minimal Selective Concentration (MSC)

- [ ] Minimal Inhibitory Concentration (MIC)
- [ ] Others
- [ ] I don't know / prefer not to answer

5.2.3.14 Please specify 'other':

100 character(s) maximum

* 5.2.3.15 Which of the following indicators currently being researched could be used to help assess the selective potency of antibiotics for environmental risk assessments?

Maximum 3 selection(s)

- [ ] Minimal concentration inducing resistance mutations
- [ ] Minimal concentration increasing the rate of horizontal transfer of mobile resistance determinants
- [ ] Minimal increased persistence concentration
- [ ] Total bacterial community growth
- [ ] Selective ability of whole effluents
- [ ] Length of time exposure to antibiotics
- [ ] ARB/ARG diversity and abundance
- [ ] Additive, synergistic or antagonistic effects of antibiotics and other chemical mixtures
- [ ] Seasonal variations of antibiotics
- [ ] Others
- [ ] I don't know / I prefer not to answer
- [ ] None of the above

5.2.3.16 Please specify 'other':

100 character(s) maximum

5.2.3.17 To which extent do you agree with the idea of using WHO's AWaRe and EMA'AMEG antibiotic prescription's prioritization schemes for selecting the antibiotics for surveillance and risk assessment of AMR levels in wastewater?

5.2.3.18 What other physico-chemical parameters would be feasible and informative to collect?

100 character(s) maximum

Methodological aspects

* 5.2.3.19 In future, which of the following methods below would be the MOST FEASIBLE AND INFORMATIVE to monitor AMR in the environment?

Maximum 10 selection(s)

- [ ] Absolute quantification of ARGs measured by qPCR

- [ ] Relative abundance of ARGs measured by qPCR
- [ ] Absolute quantification of ARGs measured by ddPCR/dPCR
- [ ] Relative abundance of ARGs measured by ddPCR/dPCR
- [ ] High-throughput real-time PCR methods (relative abundance of gene only)
- [ ] Shot-gun metagenomics
- [ ] Target enrichment metagenomics (i.e. enrichment of AMR genes in shot-metagenomics libraries)
- [ ] Quantitative isolate-based methods -phenotypic assays (such as ESBL-producing *E. coli* in Tricycle protocol)
- [ ] Quantitative isolate-based methods in combination with whole genome sequencing of isolates
- [ ] Isolate-based methods - proportion of resistant bacteria among isolates of a given species
- [ ] A combination of culture-based, qPCR and metagenomics
- [ ] A combination of culture-based methods and qPCR
- [ ] A combination of qPCR and metagenomics
- [ ] A combination of culture-based methods and metagenomics
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.2.3.20 Please specify 'other':

100 character(s) maximum

* 5.2.3.21 What should be the preferred unit reported for absolute quantitative-based assays ?

Maximum 2 selection(s)

- [ ] Cumulative sum of all quantified ARG (e.g., ARGs/L)
- [ ] Gene copies / CFU per L
- [ ] Gene copies / CFU per g of dry mass (for solids)
- [ ] Gene copies / CFU per g of wet mass (for solids)
- [ ] Gene copies / CFU per g of ashes (for solids)
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.2.3.22 Please specify 'other':

50 character(s) maximum

* 5.2.3.23 What should be the preferred relative abundance normalization method for culture-based and PCR assays?

Maximum 2 selection(s)

- [ ] *16S* rRNA gene copy number
- [ ] total DNA
- [ ] a collection of single-copy genes
- [ ] *rpoB* gene
- [ ] for culturing: concentration of non-resistant *E. coli*
- [ ] None
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.2.3.24 Please specify 'other':

50 character(s) maximum

* 5.2.3.25 What should be the preferred relative abundance normalization method for sequencing-based methods?

Maximum 2 selection(s)

- ☐ *16S* rRNA
- ☐ a collection of single copy genes
- ☐ *FPKM*
- ☐ *TPM*
- ☐ *rpoB* gene
- ☐ spike-ins of strains at known concentration
- ☐ None
- ☐ Other
- ☐ I don't know /I prefer not to answer

5.2.3.26 Please specify 'other':

50 character(s) maximum

5.2.3.27 Would there be fundamental changes to your answers on 'indicators' if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.3 Future surveillance - Surface/Ground water-based surveillance

In this section, when we speak about 'environment' or 'environmental surveillance' we mean SURFACE AND/OR GROUNDWATER surveillance. Please keep that in mind when answering.

5.3.1 Future surveillance - Objectives of surveillance

5.3.1.1 **General objectives**

Which of the following general objectives should be addressed by AMR surveillance in the environment? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer

* Provide information about patterns and trends in AMR (including identification of emission sources)							
* Support and inform risk assessment of AMR in the environment (including informing exposure assessments)							
* Alert on emergence and evolution of AMR							
* Assess the effectiveness of interventions							

5.3.1.2 **Specific objectives– Patterns and trends in AMR**

If environmental AMR surveillance should provide information about patterns and trends in AMR, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Determine spatial variations in the levels of AB /ARB/ARGs within the area under surveillance							
* Determine temporal variations in the levels of AB/ARB/ARGs within the area under surveillance							
* Identify and monitor transmission of clinically relevant AMR mechanisms across geographical borders							
* Identify and monitor sources of emissions of AMR (from humans and/or animals) as they contaminate the environment							

5.3.1.3 **Specific objectives– data for risk assessments**

If environmental AMR surveillance should deliver data to inform risk assessments, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to human health (e.g. data enabling determination of human exposure to AMR in the environment)							

* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to animal health							
* Use environmental surveillance to generate data helping to assess the risks of AMR, including antibiotics and related pollutants, to environmental health (such as to aquatic life)							
* Identify and monitor transmission of clinically relevant AMR mechanisms from humans to animals and from animals to humans							
* Apply environmental surveillance for tracing of outbreaks							
* Apply environmental surveillance to assess environmental law offences							

5.3.1.4 Other ?

100 character(s) maximum

5.3.1.5 **Specific objectives - Emergence and evolution of AMR**

If emerging forms of AMR should be monitored within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Generate data on the emergence of new clinically relevant resistance mechanisms							
* Identify and assess hotspots for emergence, genetic transmission and evolution of AMR							
* Identify and monitor predictors and drivers of AMR diversity and abundance							
* Identify and monitor transmission of clinically relevant AMR mechanisms across bacterial species							

5.3.1.6 Other ?

100 character(s) maximum

5.3.1.7 **Specific objectives – Efficiency of interventions**

If determining the efficiency of interventions to curb AMR should be pursued within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Evaluate treatment methods for AMR removal at centralised wastewater utilities	◯	◯	◯	◯	◯	◯	◯
* Assess the resilience of the environment after the implementation of reduction measures	◯	◯	◯	◯	◯	◯	◯
* Evaluate treatment methods for AMR removal at hospital wastewater facilities	◯	◯	◯	◯	◯	◯	◯
* Evaluate treatment methods for AMR removal during sludge treatment	◯	◯	◯	◯	◯	◯	◯
* Evaluate AMR removal methods during water reuse treatment for crop irrigation	◯	◯	◯	◯	◯	◯	◯

5.3.1.8 Other ?

100 character(s) maximum

Signals for urgently actionable public health interventions

In the field of public health surveillance of communicable diseases, some health threats require urgent action among competent national authorities in order to initiate a response (e.g. COVID-19). With a view to ascertain which AMR signals should require such prompt response, if any, a series of scenarios have been listed in the question below.

5.3.1.9 Which of the following AMR signals in the environment should trigger URGENTLY ACTIONABLE INTERVENTIONS? (1=most important)

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Increase in the levels of clinically relevant ARB/ARGs	◯	◯	◯	◯	◯	◯	◯
* Increase in the levels of any antibiotic	◯	◯	◯	◯	◯	◯	◯
* Increase in the levels of antibiotic residues	◯	◯	◯	◯	◯	◯	◯

* Transmission of clinically relevant AMR mechanisms in water environments across geographical borders							
* Persistence in time of AMR determinants							
* Emergence of new clinically relevant resistance mechanisms							
* Increase of AMR drivers (antibiotics, biocides, heavy metals, microplastics, etc)							
* Increase in biomarker indicators of transmissibility of ARGs (ex. mobile genetic elements)							
* Transmission of clinically relevant AMR mechanisms in water environments across human-animal species							
* Transmission of clinically relevant AMR mechanisms in water environments across bacterial species							
* Increase in the levels of AMR-related toxicity (e.g. water biodiversity loss)							

5.3.1.10 Other ?

100 character(s) maximum

5.3.1.11 Would there be fundamental changes to your answers on 'objectives' if you were to consider the far future (10 years) instead of the next 5 years? if so, please explain:

300 character(s) maximum

5.3.2 Future surveillance - Sampling

Sites

5.3.2.1 **Surface/ground water environments**

In future, which of the following water environments is essential to monitor for AMR?:

between 1 and 2 choices

- Inland water at river basins, including streams, rivers and lakes
- Transitional water, including river mouths, estuaries and deltas
- Coastal water, including wetlands, swamps and marshes
- Ground water (e.g. aquifer)
- Marine water (at least one mile away from the coastline)

- [ ] Glacier, permafrost, snow
- [ ] Storm water (e.g. from rain, hail)
- [ ] Other

5.3.2.2 Please specify 'other':

100 character(s) maximum

Frequency

5.3.2.3 In future, how frequently should the surveillance be undertaken?

- ( ) Once annually
- ( ) Twice a year
- ( ) Three times a year
- ( ) Every Three months
- ( ) Every month
- ( ) Daily
- ( ) Real time

* 5.3.2.4 In future, to which of the following NATURAL phenomena should environmental AMR surveillance adapt in order to be more representative? (1-3 answers if needed)

Maximum 3 selection(s)

- [ ] Distribution across all seasons
- [ ] Cold seasons
- [ ] Hot seasons
- [ ] The first rainfall of a season
- [ ] Events of heavy rainfall and storm overflow
- [ ] Drought period
- [ ] Sand and dust storms
- [ ] Extreme weather disasters (e.g earthquakes, volcanic eruptions, hurricanes, tsunamis)
- [ ] Bird/fish migration season
- [ ] Pollination season
- [ ] None of the above
- [ ] Other
- [ ] I don't know / prefer not to answer

5.3.2.5 Please specify 'other':

100 character(s) maximum

* 5.3.2.6 In future, to which of the following periods impacted by human activities should environmental AMR surveillance in surface/groundwater adapt to in order to be most representative? (One answer - 3 max if necessary)

Maximum 3 selection(s)

- [ ] Pesticide application periods

- [ ] Food harvest periods
- [ ] Events of treated industrial emissions
- [ ] Periods of increased incidence in human infections (e.g. respiratory disease period in winter, gastroenteritis in summer)
- [ ] Periods of increased incidence in infectious outbreaks across livestock and aquaculture
- [ ] Periods of increased incidence in pests in agriculture
- [ ] Periods of touristic travelling
- [ ] Bathing season
- [ ] Mass gatherings: religious pilgrimages (Hajj), music, sport and festivity events (Olympics, Oktoberfest)
- [ ] None of the above
- [ ] Other
- [ ] I don't know / prefer not to answer

5.3.2.7 Please specify 'other':

100 character(s) maximum

The role of AMR in global health and climate change

5.3.2.8 In your opinion, will climate change influence AMR in such a way that this should be taken into consideration for AMR surveillance in surface/groundwater, if so, please indicate how.

500 character(s) maximum

* 5.3.2.9 In your opinion, which of the following future scenarios impacted by human activities should AMR surveillance in surface/groundwater adapt to in order to be more representative?

- [ ] CBRN incidents (chemical, biological, radiological and nuclear)
- [ ] Periods of armed conflicts, war and mass casualties
- [ ] Mass migration across geographical borders
- [ ] Direct non-treated industrial discharges into the environment (e.g. shipwrecks emitting industrial substances into marine waters, leakages from pharmaceutical or hospital facilities into rivers, etc)
- [ ] Outbreaks derived from high-containment laboratories for high threat pathogens (e.g. biosafety level-4 laboratories, gain-of-function research labs)
- [ ] Severe foodborne illness/outbreaks events (e.g. *E. coli* O104:H4 outbreak)
- [ ] During any ongoing public health emergency of international concern
- [ ] None of the above
- [ ] I don't know /I prefer not to answer
- [ ] Other

5.3.2.10 Please specify 'other':

50 character(s) maximum

5.3.2.11 Would there be fundamental changes to your answers on 'sampling', ie sites and frequency, if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.3.3 Future surveillance - AMR indicators

* 5.3.3.1 **Identifying and monitoring antibiotic resistant bacteria (ARB)**

Which of the following bacterial targets would be the MOST FEASIBLE AND INFORMATIVE option for culture-based environmental surveillance of AMR?

- ☐ Aeromonas spp
- ☐ *Acinetobacter baumanii*
- ☐ *Bordetella pertussis*
- ☐ Campylobacter spp
- ☐ *Citrobacter freundii*
- ☐ *Corynebacterium diphteriae*
- ☐ *Clostridium perfringens*
- ☐ *Clostridioides difficile*
- ☐ Enterococcus spp
- ☐ *Escherichia coli*
- ☐ Shiga toxin/verocytotoxin-producing *Escherichia coli*
- ☐ *Haemophilus influenzae*
- ☐ *Klebsiella pneumonia*
- ☐ *Listeria monocytogenes*
- ☐ Legionella spp
- ☐ *Neisseria gonorrhoeae*
- ☐ *Neisseria meningitidis*
- ☐ *Pseudomonas aeruginosa*
- ☐ Salmonella spp
- ☐ Shigella spp
- ☐ *Staphylococcus aureus*
- ☐ *Streptococcus pneumoniae*
- ☐ *Vibrio cholera*
- ☐ Total coliform bacteria
- ☐ Others
- ☐ I don't know/I prefer not to answer

5.3.3.2 Please specify 'other':

100 character(s) maximum

* 5.3.3.3 Which of the following antibiotic-resistant groups of bacterial human pathogens from the WHO Bacterial Priority Pathogens list 2024 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR?

Maximum 15 selection(s)

- ☐ *Acinetobacter baumannii* carbapenem-resistant
- ☐ Enterobacterales third-generation cephalosporin-resistant
- ☐ Enterobacterales carbapenem-resistant
- ☐ *Enterococcus faecium* vancomycin-resistant
- ☐ *Haemophilus influenzae* ampicillin-resistant
- ☐ *Mycobacterium tuberculosis* rifampicin-resistant
- ☐ *Neisseria gonorrhoeae* third-generation cephalosporin, and/or fluoroquinolone-resistant
- ☐ *Pseudomonas aeruginosa* carbapenem-resistant
- ☐ *Salmonella Typhi* fluoroquinolone-resistant
- ☐ Non-typhoidal Salmonella fluoroquinolone-resistant
- ☐ Shigella spp. fluoroquinolone-resistant
- ☐ *Staphylococcus aureus* methicillin-resistant Group A
- ☐ Streptococci macrolide-resistant
- ☐ *Streptococcus pneumoniae* macrolide-resistant Group B
- ☐ Streptococci penicillin-resistant
- ☐ Others
- ☐ I don't know / prefer not to answer

5.3.3.4 Please specify 'other':

100 character(s) maximum

* 5.3.3.5 Which of the following fungal human pathogens from the WHO fungal priority pathogens list 2022 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR surveillance?

Maximum 15 selection(s)

- ☐ *Aspergillus fumigatus*
- ☐ *Candida albicans*
- ☐ *Candida auris*
- ☐ *Candida parapsilosis*
- ☐ *Candida tropicalis*
- ☐ *Coccidioides spp*
- ☐ *Cryptococcus gattii*
- ☐ *Cryptococcus neoformans*
- ☐ *Eumycetoma* causative agents
- ☐ Fusarium spp
- ☐ Histoplasma spp
- ☐ *Lomentospora prolificans*
- ☐ Mucorales
- ☐ *Nakaseomyces glabrata (Candida glabrata)*
- ☐ Paracoccidioides spp
- ☐ *Pichia kudriavzeveii (Candida krusei)*
- ☐ Pneumocystis jirovecii
- ☐ Scedosporium spp
- ☐ *Talaromyces marneffei*
- ☐ Others

- [ ] I don't know /I prefer not to answer

5.3.3.6 Other:

100 character(s) maximum

Identifying and monitoring antibiotic resistant genes (ARGs)

According to NCBI's bacterial genomic data, the highest diversity of submitted AMR gene sequences corresponds to resistance to beta-lactams, followed by aminoglycosides, quinolones, glycopeptides, tetracyclines and macrolides, among others:

* 5.3.3.7 Which of the following antibiotic resistance classes should be prioritized for the purpose of AMR surveillance in surface/groundwater?

Maximum 15 selection(s)

- [ ] Aminoglycoside resistance
- [ ] Amphenicol resistance
- [ ] Carbapenem and monobactam resistance
- [ ] Cephalosporins (First- and second-generation) resistance
- [ ] Cephalosporins (Third- and/or fourth-generation) resistance
- [ ] Fluoroquinolone resistance
- [ ] Glycopeptide resistance
- [ ] Imidazole derivative resistance
- [ ] Lincosamide and Streptogramin resistance
- [ ] Macrolide resistance
- [ ] Penicillin resistance
- [ ] Polymyxin resistance
- [ ] Tetracycline resistance
- [ ] Trimethoprim/sulphonamide resistance
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.3.3.8 Other:

100 character(s) maximum

5.3.3.9 For each of the indicator purposes shown in the table below, which antimicrobial resistance gene targets would be the best for qPCR-based AMR surveillance in surface/groundwater? Choose up to 10 genes for each category. You can use the genes shown in table 3 in the annex 1 or add other genes you might know.

	AMR proxy / anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

5.3.3.10 How would you allocate the following types of gene indicators across environmental compartmens for AMR surveillance and risk assessment purposes?

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Surface water	☐	☐	☐	☐	☐
Ground water	☐	☐	☐	☐	☐
Wastewater	☐	☐	☐	☐	☐
Water reuse	☐	☐	☐	☐	☐
Sludge (WWTP)	☐	☐	☐	☐	☐
Manure	☐	☐	☐	☐	☐
Soil	☐	☐	☐	☐	☐

5.3.3.11 How would you allocate the following types of gene indicators by the following objectives of AMR surveillance in surface/groundwater?

The above used objectives were:
1) Provide information about patterns and trends in AMR (including identification of emission sources)
2) Support and inform risk analysis of AMR in the environment
3) Alert on emergence and evolution of AMR
4) Assess the effectiveness of interventions

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Monitor trends of AMR	☐	☐	☐	☐	☐
Provide data for risk assessment	☐	☐	☐	☐	☐
Determine emerging AMR forms	☐	☐	☐	☐	☐
Evaluate interventions	☐	☐	☐	☐	☐

5.3.3.12 ***Identifying and monitoring levels of antibiotic and antibiotic residues***

Which of the following antimicrobial classes should be monitored for AMR risk assessment in surface /groundwater?

Antibiotics	Antibiotics *Maximum 15 selection(s)* ☐ Aminoglycosides ☐ Amphenicols ☐ Carbapenems and monobactams ☐ First- and second-generation cephalosporins ☐ Fluoroquinolones ☐ Glycopeptides ☐ Imidazole derivatives ☐ Lincosamides and Streptogramins ☐ Macrolides ☐ Other ☐ Penicillins ☐ Polymyxins ☐ Tetracyclines ☐ Third- and/or fourth-generation cephalosporins ☐ Trimethoprim/sulphonamides
Antimycotics	Antimycotics *Maximum 5 selection(s)* ☐ Azoles ☐ Amphotericin B ☐ Echinocandins ☐ Terbinafine ☐ Others
Disinfectants / preservatives	Disinfectants / preservatives ☐ Triclosan ☐ Quaternary ammonium compounds QACs ☐ Chlorhexidine ☐ Chlorine-releasing compounds ☐ Aldehyde-based compounds ☐ Alcohols ☐ Hydrogen peroxide ☐ Peracetic acid ☐ Weak organic acids ☐ Others

* 5.3.3.13 **Defining criteria for risk assessment of antibiotics and antibiotic residues**

Which of the following already established indicators should be used to assess the selective potency of antibiotics for environmental risk assessments?

- ☐ Lowest Observed Effect Concentration (LOEC)
- ☐ No Observed Effect Concentration (NOEC)
- ☐ Predicted No Effect Concentration (PNEC)
- ☐

Minimal Selective Concentration (MSC)

- [ ] Minimal Inhibitory Concentration (MIC)
- [ ] Others
- [ ] I don't know / prefer not to answer

5.3.3.14 Please specify 'other':

100 character(s) maximum

* 5.3.3.15 Which of the following indicators currently being researched could be used to help assess the selective potency of antibiotics for environmental risk assessments?

Maximum 3 selection(s)

- [ ] Minimal concentration inducing resistance mutations
- [ ] Minimal concentration increasing the rate of horizontal transfer of mobile resistance determinants
- [ ] Minimal increased persistence concentration
- [ ] Total bacterial community growth
- [ ] Selective ability of whole effluents
- [ ] Length of time exposure to antibiotics
- [ ] ARB/ARG diversity and abundance
- [ ] Additive, synergistic or antagonistic effects of antibiotics and other chemical mixtures
- [ ] Seasonal variations of antibiotics
- [ ] Others
- [ ] I don't know / prefer not to answer
- [ ] None of the above

5.3.3.16 Please specify 'other':

100 character(s) maximum

5.3.3.17 To which extent do you agree with the idea of using WHO's AWaRe and EMA'AMEG antibiotic prescription's prioritization schemes for selecting the antibiotics for surveillance and risk assessment of AMR levels in surface/groundwater?

5.3.3.18 ***Identifying and monitoring levels of AMR drivers***

In future, which of the following natural physico-chemical parameters would be the MOST FEASIBLE AND INFORMATIVE metadata of AMR to monitor in surface/groundwater?

- [ ] Temperature
- [ ] Oxygenation conditions
- [ ] Salinity
- [ ] pH
- [ ] Nutrient conditions
- [ ] Hydrodynamic conditions
- [ ]

Others

- [ ] None of the above

5.3.3.19 What other physico-chemical parameters would be feasible and informative to collect?

100 character(s) maximum

Methodological aspects

* 5.3.3.20 In future, which of the following methods below would be the MOST FEASIBLE AND INFORMATIVE to monitor AMR in surface/groundwater?

Maximum 10 selection(s)

- [ ] Absolute quantification of ARGs measured by qPCR
- [ ] Relative abundance of ARGs measured by qPCR
- [ ] Absolute quantification of ARGs measured by ddPCR/dPCR
- [ ] Relative abundance of ARGs measured by ddPCR/dPCR
- [ ] High-throughput real-time PCR methods (relative abundance of gene only)
- [ ] Shot-gun metagenomics
- [ ] Target enrichment metagenomics (i.e. enrichment of AMR genes in shot-metagenomics libraries)
- [ ] Quantitative isolate-based methods -phenotypic assays (such as ESBL-producing *E. coli* in Tricycle protocol)
- [ ] Quantitative isolate-based methods in combination with whole genome sequencing of isolates
- [ ] Isolate-based methods - proportion of resistant bacteria among isolates of a given species
- [ ] A combination of culture-based, qPCR and metagenomics
- [ ] A combination of culture-based methods and qPCR
- [ ] A combination of qPCR and metagenomics
- [ ] A combination of culture-based methods and metagenomics
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.3.3.21 Please specify 'other':

100 character(s) maximum

* 5.3.3.22 What should be the preferred unit reported for absolute quantitative-based assays ?

Maximum 2 selection(s)

- [ ] Cumulative sum of all quantified ARG (e.g., ARGs/L)
- [ ] Gene copies / CFU per L
- [ ] Gene copies / CFU per g of dry mass (for solids)
- [ ] Gene copies / CFU per g of wet mass (for solids)
- [ ] Gene copies / CFU per g of ashes (for solids)
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.3.3.23 Please specify 'other':

50 character(s) maximum

* 5.3.3.24 What should be the preferred relative abundance normalization method for culture-based and PCR assays?

Maximum 2 selection(s)

- *16S* rRNA gene copy number
- total DNA
- a collection of single-copy genes
- *rpoB* gene
- for culturing: concentration of non-resistant *E. coli*
- None
- Other
- I don't know /I prefer not to answer

5.3.3.25 Please specify 'other':

50 character(s) maximum

* 5.3.3.26 What should be the preferred relative abundance normalization method for sequencing-based methods?

Maximum 2 selection(s)

- *16S* rRNA
- a collection of single copy genes
- *FPKM*
- *TPM*
- *rpoB* gene
- spike-ins of strains at known concentration
- None
- Other
- I don't know /I prefer not to answer

5.3.3.27 Please specify 'other':

50 character(s) maximum

5.3.3.28 Would there be fundamental changes to your answers on 'indicators' if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.4 Future surveillance - Soil and/or related environments, biosolids and irrigation water

In this section, when we speak about 'environment' or 'environmental surveillance' we mean SOIL, BIOSOLIDS AND/OR IRRIGATION WATER (surveillance). Please keep that in mind when answering.

5.4.1 Future surveillance - Objectives of surveillance

5.4.1.1 **General objectives**

Which of the following general objectives should be addressed by AMR surveillance in the environment? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Provide information about patterns and trends in AMR (including identification of emission sources)	○	○	○	○	○	○	○
* Support and inform risk assessment of AMR in the environment (including informing exposure assessments)	○	○	○	○	○	○	○
* Alert on emergence and evolution of AMR	○	○	○	○	○	○	○
* Assess the effectiveness of interventions	○	○	○	○	○	○	○

5.4.1.2 **Specific objectives– Patterns and trends in AMR**

If environmental AMR surveillance should provide information about patterns and trends in AMR, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Determine spatial variations in the levels of AB /ARB/ARGs within the area under surveillance	○	○	○	○	○	○	○
* Determine temporal variations in the levels of AB/ARB/ARGs within the area under surveillance	○	○	○	○	○	○	○
* Identify and monitor transmission of clinically relevant AMR mechanisms across geographical borders	○	○	○	○	○	○	○

* Identify and monitor sources of emissions of AMR (from humans and/or animals) as they contaminate the environment							

5.4.1.3 **Specific objectives– data for risk assessments**

If environmental AMR surveillance should deliver data to inform risk assessments, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to human health (e.g. data enabling determination of human exposure to AMR in the environment)							
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to animal health							
* Use environmental surveillance to generate data helping to assess the risks of AMR, including antibiotics and related pollutants, to environmental health (such as to aquatic life)							
* Identify and monitor transmission of clinically relevant AMR mechanisms from humans to animals and from animals to humans							
* Apply environmental surveillance to assess environmental law offences							

5.4.1.4 Other ?

100 character(s) maximum

5.4.1.5 **Specific objectives - Emergence and evolution of AMR**

If emerging forms of AMR should be monitored within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer

* Generate data on the emergence of new clinically relevant resistance mechanisms	○	○	○	○	○	○	○
* Identify and assess hotspots for emergence, genetic transmission and evolution of AMR	○	○	○	○	○	○	○
* Identify and monitor predictors and drivers of AMR diversity and abundance	○	○	○	○	○	○	○
* Identify and monitor transmission of clinically relevant AMR mechanisms across bacterial species	○	○	○	○	○	○	○

5.4.1.6 Other ?

100 character(s) maximum

5.4.1.7 **Specific objectives – Efficiency of interventions**

If determining the efficiency of interventions to curb AMR should be pursued within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Evaluate treatment methods for AMR removal during manure treatment	○	○	○	○	○	○	○
* Evaluate the efficiency of changes in antibiotic stewardship and/or infection prevention and control within human or animal populations	○	○	○	○	○	○	○
* Assess the resilience of the environment after the implementation of reduction measures	○	○	○	○	○	○	○
* Evaluate AMR removal methods during water reuse treatment for crop irrigation	○	○	○	○	○	○	○
* Evaluate the environmental safety and applicability of soil fertilization with treated sludge/manure in relation to AMR	○	○	○	○	○	○	○
* Evaluate the efficiency of changes in pesticide application methods onto agricultural soils for plant health and soil biodiversity risk assessments in relation to AMR	○	○	○	○	○	○	○

5.4.1.8 Other ?

100 character(s) maximum

Signals for urgently actionable public health interventions

In the field of public health surveillance of communicable diseases, some health threats require urgent action among competent national authorities in order to initiate a response (e.g. COVID-19). With a view to ascertain which AMR signals should require such prompt response, if any, a series of scenarios have been listed in the question below.

5.4.1.9 Which of the following AMR signals in the environment should trigger URGENTLY ACTIONABLE INTERVENTIONS? (1=most important)

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Increase in the levels of clinically relevant ARB/ARGs	○	○	○	○	○	○	○
* Increase in the levels of any antibiotic	○	○	○	○	○	○	○
* Increase in the levels of antibiotic residues	○	○	○	○	○	○	○
* Transmission of clinically relevant AMR mechanisms in water/soil environments across geographical borders	○	○	○	○	○	○	○
* Persistence in time of AMR determinants	○	○	○	○	○	○	○
* Emergence of new clinically relevant resistance mechanisms	○	○	○	○	○	○	○
* Increase of AMR drivers (antibiotics, biocides, heavy metals, microplastics, etc)	○	○	○	○	○	○	○
* Increase in biomarker indicators of transmissibility of ARGs (ex. mobile genetic elements)	○	○	○	○	○	○	○
* Transmission of clinically relevant AMR mechanisms in water/soil environments across human-animal species	○	○	○	○	○	○	○
* Transmission of clinically relevant AMR mechanisms in water/soil environments across bacterial species	○	○	○	○	○	○	○
* Increase in the levels of AMR-related toxicity (e.g. water/soil biodiversity loss)	○	○	○	○	○	○	○

5.4.1.10 Other ?

100 character(s) maximum

5.4.1.11 Would there be fundamental changes to your answers on 'objectives' if you were to consider the far future (10 years) instead of the next 5 years? if so, please explain:

300 character(s) maximum

5.4.2 Future surveillance - Sampling

Sites

5.4.2.1 **Soil (related) environments, biosolids and irrigation water**

In future, which of the following soil-related environments is essential to monitor AMR?

If you think of a compartment not included in this list, please check the land cover and land use Copernicus nomenclature to confirm your compartment is not already included in the ontology.

between 1 and 5 choices

- ☐ Agricultural soils
- ☐ Wetlands
- ☐ Urban soils
- ☐ Artificial soils
- ☐ Pasture
- ☐ Forest and semi natural soil
- ☐ Soils irrigated with re-used water
- ☐ Soils fertilized with manure/sludge etc.
- ☐ Wastewater sludge
- ☐ Manure for fertilization
- ☐ Irrigation water
- ☐ Other

5.4.2.2 Please specify 'other':

50 character(s) maximum

Frequency

5.4.2.3 In future, how frequently should the surveillance be undertaken?

- ○ Once annually
- ○ Twice a year
- ○ Three times a year

- Every Three months
- Every month
- Daily
- Real time

* 5.4.2.4 In future, to which of the following NATURAL phenomena should AMR surveillance in soil and/or related environments adapt in order to be most representative? (1-3 answers if needed)

Maximum 3 selection(s)

- Distribution across all seasons
- Cold seasons
- Hot seasons
- The first rainfall of a season
- Events of heavy rainfall and storm overflow
- Drought period
- Sand and dust storms
- Extreme weather disasters (e.g earthquakes, volcanic eruptions, hurricanes, tsunamis)
- Bird/fish migration season
- Pollination season
- None of the above
- Other
- I don't know / prefer not to answer

5.4.2.5 Please specify 'other':

100 character(s) maximum

* 5.4.2.6 In future, to which of the following periods impacted by human activities should AMR surveillance in soil and/or related environments adapt to in order to be most representative? (One answer - 3 max if necessary)

Maximum 3 selection(s)

- Pesticide application periods
- Food harvest periods
- Events of treated industrial emissions
- Periods of increased incidence in human infections (e.g. respiratory disease period in winter, gastroenteritis in summer)
- Periods of increased incidence in infectious outbreaks across livestock and aquaculture
- Periods of increased incidence in pests in agriculture
- Periods of touristic travelling
- Bathing season
- Mass gatherings: religious pilgrimages (Hajj), music, sport and festivity events (Olympics, Oktoberfest)
- None of the above
- Other
- I don't know / prefer not to answer

5.4.2.7 Please specify 'other':

100 character(s) maximum

The role of AMR in global health and climate change

5.4.2.8 In your opinion, will climate change influence AMR in such a way that this should be taken into consideration for AMR surveillance in soil and/or related environments, if so, please indicate how.

500 character(s) maximum

* 5.4.2.9 In your opinion, which of the following future scenarios impacted by human activities should AMR surveillance in soil and/or related environments adapt to in order to be more representative?

- CBRN incidents (chemical, biological, radiological and nuclear)
- Periods of armed conflicts, war and mass casualties
- Mass migration across geographical borders
- Direct non-treated industrial discharges into the environment (e.g. shipwrecks emitting industrial substances into marine waters, leakages from pharmaceutical or hospital facilities into rivers, etc)
- Outbreaks derived from high-containment laboratories for high threat pathogens (e.g. biosafety level-4 laboratories, gain-of-function research labs)
- Severe foodborne illness/outbreaks events (e.g. *E. coli* O104:H4 outbreak)
- During any ongoing public health emergency of international concern
- None of the above
- I don't know /I prefer not to answer
- Other

5.4.2.10 Please specify 'other':

50 character(s) maximum

5.4.2.11 Would there be fundamental changes to your answers on 'sampling', ie sites and frequency, if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.4.3 Future surveillance - AMR indicators

* 5.4.3.1 **Identifying and monitoring antibiotic resistant bacteria (ARB)**

Which of the following bacterial targets would be the MOST FEASIBLE AND INFORMATIVE option for culture-based surveillance of AMR?

- Aeromonas spp
- *Acinetobacter baumanii*
- *Bordetella pertussis*
- Campylobacter spp

- ☐ *Citrobacter freundii*
- ☐ *Corynebacterium diphteriae*
- ☐ *Clostridium perfringens*
- ☐ *Clostridioides difficile*
- ☐ Enterococcus spp
- ☐ *Escherichia coli*
- ☐ Shiga toxin/verocytotoxin-producing *Escherichia coli*
- ☐ *Haemophilus influenzae*
- ☐ *Klebsiella pneumonia*
- ☐ *Listeria monocytogenes*
- ☐ Legionella spp
- ☐ *Neisseria gonorrhoeae*
- ☐ *Neisseria meningitidis*
- ☐ *Pseudomonas aeruginosa*
- ☐ Salmonella spp
- ☐ Shigella spp
- ☐ *Staphylococcus aureus*
- ☐ *Streptococcus pneumoniae*
- ☐ *Vibrio cholera*
- ☐ Total coliform bacteria
- ☐ Others
- ☐ I don't know/I prefer not to answer

5.4.3.2 Please specify 'other':

100 character(s) maximum

* 5.4.3.3 Which of the following antibiotic-resistant groups of bacterial human pathogens from the WHO Bacterial Priority Pathogens list 2024 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR?

Maximum 15 selection(s)

- ☐ *Acinetobacter baumannii* carbapenem-resistant
- ☐ Enterobacterales third-generation cephalosporin-resistant
- ☐ Enterobacterales carbapenem-resistant
- ☐ *Enterococcus faecium* vancomycin-resistant
- ☐ *Haemophilus influenzae* ampicillin-resistant
- ☐ *Mycobacterium tuberculosis* rifampicin-resistant
- ☐ *Neisseria gonorrhoeae* third-generation cephalosporin, and/or fluoroquinolone-resistant
- ☐ *Pseudomonas aeruginosa* carbapenem-resistant
- ☐ *Salmonella Typhi* fluoroquinolone-resistant
- ☐ Non-typhoidal Salmonella fluoroquinolone-resistant
- ☐ Shigella spp. fluoroquinolone-resistant
- ☐ *Staphylococcus aureus* methicillin-resistant Group A
- ☐ Streptococci macrolide-resistant
- ☐ *Streptococcus pneumoniae* macrolide-resistant Group B
- ☐ Streptococci penicillin-resistant

- [ ] Others
- [ ] I don't know / prefer not to answer

5.4.3.4 Please specify 'other':

100 character(s) maximum

* 5.4.3.5 Which of the following fungal human pathogens from the WHO fungal priority pathogens list 2022 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR surveillance?

Maximum 15 selection(s)

- [ ] *Aspergillus fumigatus*
- [ ] *Candida albicans*
- [ ] *Candida auris*
- [ ] *Candida parapsilosis*
- [ ] *Candida tropicalis*
- [ ] *Coccidioides spp*
- [ ] *Cryptococcus gattii*
- [ ] *Cryptococcus neoformans*
- [ ] *Eumycetoma* causative agents
- [ ] Fusarium spp
- [ ] Histoplasma spp
- [ ] *Lomentospora prolificans*
- [ ] Mucorales
- [ ] *Nakaseomyces glabrata (Candida glabrata)*
- [ ] Paracoccidioides spp
- [ ] *Pichia kudriavzeveii (Candida krusei)*
- [ ] Pneumocystis jirovecii
- [ ] Scedosporium spp
- [ ] *Talaromyces marneffei*
- [ ] Others
- [ ] I don't know /I prefer not to answer

5.4.3.6 Other:

100 character(s) maximum

Identifying and monitoring antibiotic resistant genes (ARGs)

According to NCBI's bacterial genomic data, the highest diversity of submitted AMR gene sequences corresponds to resistance to beta-lactams, followed by aminoglycosides, quinolones, glycopeptides, tetracyclines and macrolides, among others.

* 5.4.3.7 Which of the following antibiotic resistance classes should be prioritized for the purpose of AMR surveillance in soil and/or related environments?

Maximum 15 selection(s)

- [ ] Aminoglycoside resistance
- [ ] Amphenicol resistance
- [ ] Carbapenem and monobactam resistance
- [ ] Cephalosporins (First- and second-generation) resistance
- [ ] Cephalosporins (Third- and/or fourth-generation) resistance
- [ ] Fluoroquinolone resistance
- [ ] Glycopeptide resistance
- [ ] Imidazole derivative resistance
- [ ] Lincosamide and Streptogramin resistance
- [ ] Macrolide resistance
- [ ] Penicillin resistance
- [ ] Polymyxin resistance
- [ ] Tetracycline resistance
- [ ] Trimethoprim/sulphonamide resistance
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.4.3.8 Other:

100 character(s) maximum

5.4.3.9 For each of the indicator purposes shown in the table below, which antimicrobial resistance gene targets would be the best for qPCR-based AMR surveillance in soil and/or related environments? Choose up to 10 genes for each category. You can use the genes shown in table 3 in the annex 1 or add other genes you might know.

	AMR proxy / anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

5.4.3.10 How would you allocate the following types of gene indicators across environmental compartments for AMR surveillance and risk assessment purposes?

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Surface water	☐	☐	☐	☐	☐
Ground water	☐	☐	☐	☐	☐
Wastewater	☐	☐	☐	☐	☐
Water reuse	☐	☐	☐	☐	☐
Sludge (WWTP)	☐	☐	☐	☐	☐
Manure	☐	☐	☐	☐	☐
Urban and/or artificial soils	☐	☐	☐	☐	☐
Agricultural soils and/or pastures	☐	☐	☐	☐	☐
Forests and seminatural areas	☐	☐	☐	☐	☐
Wetlands	☐	☐	☐	☐	☐

5.4.3.11 How would you allocate the the following types of gene indicators by the following objectives of AMR surveillance in soil and/or related environments?

The above used objectives were:
1) Provide information about patterns and trends in AMR (including identification of emission sources)
2) Support and inform risk analysis of AMR in the environment
3) Alert on emergence and evolution of AMR
4) Assess the effectiveness of interventions

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Monitor trends of AMR	☐	☐	☐	☐	☐
Provide data for risk assessment	☐	☐	☐	☐	☐
Determine emerging AMR forms	☐	☐	☐	☐	☐
Evaluate interventions	☐	☐	☐	☐	☐

5.4.3.12 ***Identifying and monitoring levels of antibiotic and antibiotic residues***

Which of the following antimicrobial classes should be monitored for AMR risk assessment in soil and/or related environments?

Antibiotics	Antibiotics *Maximum 15 selection(s)* ☐ Aminoglycosides ☐ Amphenicols ☐ Carbapenems and monobactams ☐ First- and second-generation cephalosporins ☐ Fluoroquinolones ☐ Glycopeptides ☐ Imidazole derivatives ☐ Lincosamides and Streptogramins ☐ Macrolides ☐ Other ☐ Penicillins ☐ Polymyxins ☐ Tetracyclines ☐ Third- and/or fourth-generation cephalosporins ☐ Trimethoprim/sulphonamides
Antimycotics	Antimycotics *Maximum 5 selection(s)* ☐ Azoles ☐ Amphotericin B ☐ Echinocandins ☐ Terbinafine ☐ Others
Disinfectants / preservatives	Disinfectants / preservatives ☐ Triclosan ☐ Quaternary ammonium compounds QACs ☐ Chlorhexidine ☐ Chlorine-releasing compounds ☐ Aldehyde-based compounds ☐ Alcohols ☐ Hydrogen peroxide ☐ Peracetic acid ☐ Weak organic acids ☐ Others

* 5.4.3.13 **Defining criteria for risk assessment of antibiotics and antibiotic residues**

Which of the following already established indicators should be used to assess the selective potency of antibiotics for environmental risk assessments?

- ☐ Lowest Observed Effect Concentration (LOEC)
- ☐ No Observed Effect Concentration (NOEC)
- ☐ Predicted No Effect Concentration (PNEC)
- ☐

Minimal Selective Concentration (MSC)

- [ ] Minimal Inhibitory Concentration (MIC)
- [ ] Others
- [ ] I don't know / prefer not to answer

5.4.3.14 Please specify 'other':

100 character(s) maximum

* 5.4.3.15 Which of the following indicators currently being researched could be used to help assess the selective potency of antibiotics for environmental risk assessments?

Maximum 3 selection(s)

- [ ] Minimal concentration inducing resistance mutations
- [ ] Minimal concentration increasing the rate of horizontal transfer of mobile resistance determinants
- [ ] Minimal increased persistence concentration
- [ ] Total bacterial community growth
- [ ] Selective ability of whole effluents
- [ ] Length of time exposure to antibiotics
- [ ] ARB/ARG diversity and abundance
- [ ] Additive, synergistic or antagonistic effects of antibiotics and other chemical mixtures
- [ ] Seasonal variations of antibiotics
- [ ] Others
- [ ] I don't know / prefer not to answer
- [ ] None of the above

5.4.3.16 Please specify 'other':

100 character(s) maximum

5.4.3.17 To which extent do you agree with the idea of using WHO's AWaRe and EMA'AMEG antibiotic prescription's prioritization schemes for selecting the antibiotics for surveillance and risk assessment of AMR levels in soil and/or related environments?

5.4.3.18 ***Identifying and monitoring levels of AMR drivers***

In future, which of the following natural physico-chemical parameters would be the MOST FEASIBLE AND INFORMATIVE metadata of AMR to monitor in soil and/or related environments?

- [ ] Temperature
- [ ] Oxygenation conditions
- [ ] Salinity
- [ ] pH
- [ ] Nutrient conditions
- [ ] Hydrodynamic conditions
- [ ]

Others

- [ ] None of the above

5.4.3.19 What other physico-chemical parameters would be feasible and informative to collect?

100 character(s) maximum

Methodological aspects

* 5.4.3.20 In future, which of the following methods below would be the MOST FEASIBLE AND INFORMATIVE to monitor AMR in soil and/or related environments?

Maximum 10 selection(s)

- [ ] Absolute quantification of ARGs measured by qPCR
- [ ] Relative abundance of ARGs measured by qPCR
- [ ] Absolute quantification of ARGs measured by ddPCR/dPCR
- [ ] Relative abundance of ARGs measured by ddPCR/dPCR
- [ ] High-throughput real-time PCR methods (relative abundance of gene only)
- [ ] Shot-gun metagenomics
- [ ] Target enrichment metagenomics (i.e. enrichment of AMR genes in shot-metagenomics libraries)
- [ ] Quantitative isolate-based methods -phenotypic assays (such as ESBL-producing *E. coli* in Tricycle protocol)
- [ ] Quantitative isolate-based methods in combination with whole genome sequencing of isolates
- [ ] Isolate-based methods - proportion of resistant bacteria among isolates of a given species
- [ ] A combination of culture-based, qPCR and metagenomics
- [ ] A combination of culture-based methods and qPCR
- [ ] A combination of qPCR and metagenomics
- [ ] A combination of culture-based methods and metagenomics
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.4.3.21 Please specify 'other':

100 character(s) maximum

* 5.4.3.22 What should be the preferred unit reported for absolute quantitative-based assays ?

Maximum 2 selection(s)

- [ ] Cumulative sum of all quantified ARG (e.g., ARGs/L)
- [ ] Gene copies / CFU per L
- [ ] Gene copies / CFU per g of dry mass (for solids)
- [ ] Gene copies / CFU per g of wet mass (for solids)
- [ ] Gene copies / CFU per g of ashes (for solids)
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.4.3.23 Please specify 'other':

50 character(s) maximum

* 5.4.3.24 What should be the preferred relative abundance normalization method for culture-based and PCR assays?

Maximum 2 selection(s)

- *16S* rRNA gene copy number
- total DNA
- a collection of single-copy genes
- *rpoB* gene
- for culturing: concentration of non-resistant *E. coli*
- None
- Other
- I don't know /I prefer not to answer

5.4.3.25 Please specify 'other':

50 character(s) maximum

* 5.4.3.26 What should be the preferred relative abundance normalization method for sequencing-based methods?

Maximum 2 selection(s)

- *16S* rRNA
- a collection of single copy genes
- *FPKM*
- *TPM*
- *rpoB* gene
- spike-ins of strains at known concentration
- None
- Other
- I don't know /I prefer not to answer

5.4.3.27 Please specify 'other':

50 character(s) maximum

5.4.3.28 Would there be fundamental changes to your answers on 'indicators' if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.5 Future surveillance - Other compartment

In this section, when we speak about 'environment' or 'environmental surveillance' we mean THE OTHER (SUB)COMPARTMENT OF CHOICE (surveillance). Please keep that in mind when answering.

5.5.1 Future surveillance - Objectives of surveillance

5.5.1.1 **General objectives**

Which of the following general objectives should be addressed by AMR surveillance in the environment? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Provide information about patterns and trends in AMR (including identification of emission sources)	○	○	○	○	○	○	○
* Support and inform risk assessment of AMR in the environment (including informing exposure assessments)	○	○	○	○	○	○	○
* Alert on emergence and evolution of AMR	○	○	○	○	○	○	○
* Assess the effectiveness of interventions	○	○	○	○	○	○	○

5.5.1.2 **Specific objectives– Patterns and trends in AMR**

If environmental AMR surveillance should provide information about patterns and trends in AMR, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Determine spatial variations in the levels of AB /ARB/ARGs within the area under surveillance	○	○	○	○	○	○	○
* Determine temporal variations in the levels of AB/ARB/ARGs within the area under surveillance	○	○	○	○	○	○	○
* Identify and monitor transmission of clinically relevant AMR mechanisms across geographical borders	○	○	○	○	○	○	○

* Identify and monitor sources of emissions of AMR (from humans and/or animals) as they contaminate the environment							

5.5.1.3 **Specific objectives– data for risk assessments**

If environmental AMR surveillance should deliver data to inform risk assessments, for which objectives should data be collected? Rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to human health (e.g. data enabling determination of human exposure to AMR in the environment)							
* Use environmental surveillance to generate data helping to assess the risks of AMR and related pollutants to animal health							
* Use environmental surveillance to generate data helping to assess the risks of AMR, including antibiotics and related pollutants, to environmental health (such as to aquatic life)							
* Identify and monitor transmission of clinically relevant AMR mechanisms from humans to animals and from animals to humans							
* Apply environmental surveillance for tracing of outbreaks							
* Apply environmental surveillance to assess environmental law offences							

5.5.1.4 Other ?

100 character(s) maximum

5.5.1.5 **Specific objectives - Emergence and evolution of AMR**

If emerging forms of AMR should be monitored within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5		I don't know/I prefer

						Not important at all	not to answer
* Generate data on the emergence of new clinically relevant resistance mechanisms							
* Identify and assess hotspots for emergence, genetic transmission and evolution of AMR							
* Identify and monitor predictors and drivers of AMR diversity and abundance							
* Identify and monitor transmission of clinically relevant AMR mechanisms across bacterial species							

5.5.1.6 Other ?

100 character(s) maximum

5.5.1.7 **Specific objectives – Efficiency of interventions**

If determining the efficiency of interventions to curb AMR should be pursued within environmental surveillance of AMR, rank the following options in order of importance (1=most important).

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Evaluate treatment methods for AMR removal at centralised wastewater utilities							
* Evaluate treatment methods for AMR removal during manure treatment							
* Evaluate the efficiency of changes in antibiotic stewardship and/or infection prevention and control within human or animal populations							
* Assess the resilience of the environment after the implementation of reduction measures							
* Evaluate treatment methods for AMR removal at hospital wastewater facilities							
* Evaluate treatment methods for AMR removal during sludge treatment							
* Evaluate AMR removal methods during water reuse treatment for crop irrigation							

5.5.1.8 Other ?

100 character(s) maximum

Signals for urgently actionable public health interventions

In the field of public health surveillance of communicable diseases, some health threats require urgent action among competent national authorities in order to initiate a response (e.g. COVID-19). With a view to ascertain which AMR signals should require such prompt response, if any, a series of scenarios have been listed in the question below.

5.5.1.9 Which of the following AMR signals in the environment should trigger URGENTLY ACTIONABLE INTERVENTIONS? (1=most important)

	1	2	3	4	5	Not important at all	I don't know/I prefer not to answer
* Increase in the levels of clinically relevant ARB/ARGs							
* Increase in the levels of any antibiotic							
* Increase in the levels of antibiotic residues							
* Transmission of clinically relevant AMR mechanisms in water environments across geographical borders							
* Persistence in time of AMR determinants							
* Emergence of new clinically relevant resistance mechanisms							
* Increase of AMR drivers (antibiotics, biocides, heavy metals, microplastics, etc)							
* Increase in biomarker indicators of transmissibility of ARGs (ex. mobile genetic elements)							
* Transmission of clinically relevant AMR mechanisms in water environments across human-animal species							
* Transmission of clinically relevant AMR mechanisms in water environments across bacterial species							
* Increase in the levels of AMR-related toxicity (e.g. water biodiversity loss)							

5.5.1.10 Other ?

100 character(s) maximum

5.5.1.11 Would there be fundamental changes to your answers on 'objectives' if you were to consider the far future (10 years) instead of the next 5 years? if so, please explain:

300 character(s) maximum

5.5.2 Future surveillance - Sampling

Sites

5.5.2.1 Please specify the sampling site(s) for surveillance of your other environmental sub compartment of choice.

50 character(s) maximum

Frequency

5.5.2.2 In future, how frequently should the surveillance be undertaken?

- ○ Once annually
- ○ Twice a year
- ○ Three times a year
- ○ Every Three months
- ○ Every month
- ○ Daily
- ○ Real time

* 5.5.2.3 In future, to which of the following NATURAL phenomena should AMR surveillance adapt in order to be more representative? (1-3 answers if needed)

Maximum 3 selection(s)

- ☐ Distribution across all seasons
- ☐ Cold seasons
- ☐ Hot seasons
- ☐ The first rainfall of a season
- ☐ Events of heavy rainfall and storm overflow
- ☐ Drought period
- ☐ Sand and dust storms
- ☐ Extreme weather disasters (e.g earthquakes, volcanic eruptions, hurricanes, tsunamis)
- ☐ Bird/fish migration season
- ☐ Pollination season

- [ ] None of the above
- [ ] Other
- [ ] I don't know / prefer not to answer

5.5.2.4 Please specify 'other':

100 character(s) maximum

* 5.5.2.5 In future, to which of the following periods impacted by human activities should AMR surveillance adapt to in order to be most representative? (One answer - 3 max if necessary)

Maximum 3 selection(s)

- [ ] Pesticide application periods
- [ ] Food harvest periods
- [ ] Events of treated industrial emissions
- [ ] Periods of increased incidence in human infections (e.g. respiratory disease period in winter, gastroenteritis in summer)
- [ ] Periods of increased incidence in infectious outbreaks across livestock and aquaculture
- [ ] Periods of increased incidence in pests in agriculture
- [ ] Periods of touristic travelling
- [ ] Bathing season
- [ ] Mass gatherings: religious pilgrimages (Hajj), music, sport and festivity events (Olympics, Oktoberfest)
- [ ] None of the above
- [ ] Other
- [ ] I don't know / prefer not to answer

5.5.2.6 Please specify 'other':

100 character(s) maximum

The role of AMR in global health and climate change

5.5.2.7 In your opinion, will climate change influence AMR in such a way that this should be taken into consideration for AMR surveillance, if so, please indicate how.

500 character(s) maximum

* 5.5.2.8 In your opinion, which of the following future scenarios impacted by human activities should AMR surveillance adapt to in order to be more representative?

- [ ] CBRN incidents (chemical, biological, radiological and nuclear)
- [ ] Periods of armed conflicts, war and mass casualties
- [ ] Mass migration across geographical borders
- [ ] Direct non-treated industrial discharges into the environment (e.g. shipwrecks emitting industrial substances into marine waters, leakages from pharmaceutical or hospital facilities into rivers, etc)
- [ ]

- Outbreaks derived from high-containment laboratories for high threat pathogens (e.g. biosafety level-4 laboratories, gain-of-function research labs)
- Severe foodborne illness/outbreaks events (e.g. *E. coli* O104:H4 outbreak)
- During any ongoing public health emergency of international concern
- None of the above
- I don't know /I prefer not to answer
- Other

5.5.2.9 Please specify 'other':

50 character(s) maximum

* 5.5.2.10 In future, which of the following vulnerable populations should and practically can be the focus of the AMR water surveillance?

- Victims of CBRN incidents (chemical, biological, radiological and nuclear)
- War victims, displaced people, people in refugee camps
- Elderly, children and pregnant women
- Students
- Chronically ill and disabled
- Low-income, homeless individuals and children in foster care
- Rural isolated populations
- Racial or ethnic minorities (e.g. indigenous or immigrant communities)
- Institutionalized persons (for example, persons in correctional facilities, nursing homes or mental health facilities)
- People engaging in criminal activities (e.g. use of illegal substances)
- Sex workers and victims of sexual trafficking
- Employees of the industry sector (e.g. wastewater, landfill)
- Farmers
- Health professionals (e.g. medical doctors, veterinarians, nurses)
- People residing in areas with non-sewered sanitation
- People residing in areas near WWTPs
- None of the above
- Other
- I don't know /I prefer not to answer

5.5.2.11 Please specify 'other':

150 character(s) maximum

5.5.2.12 Would there be fundamental changes to your answers on 'sampling', ie sites and frequency, if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.5.3 Future surveillance - AMR indicators

* 5.5.3.1 **Identifying and monitoring antibiotic resistant bacteria (ARB)**

Which of the following bacterial targets would be the MOST FEASIBLE AND INFORMATIVE option for culture-based surveillance of AMR?

- ☐ Aeromonas spp
- ☐ *Acinetobacter baumanii*
- ☐ *Bordetella pertussis*
- ☐ Campylobacter spp
- ☐ *Citrobacter freundii*
- ☐ *Corynebacterium diphteriae*
- ☐ *Clostridium perfringens*
- ☐ *Clostridioides difficile*
- ☐ Enterococcus spp
- ☐ *Escherichia coli*
- ☐ Shiga toxin/verocytotoxin-producing *Escherichia coli*
- ☐ *Haemophilus influenzae*
- ☐ *Klebsiella pneumonia*
- ☐ *Listeria monocytogenes*
- ☐ Legionella spp
- ☐ *Neisseria gonorrhoeae*
- ☐ *Neisseria meningitidis*
- ☐ *Pseudomonas aeruginosa*
- ☐ Salmonella spp
- ☐ Shigella spp
- ☐ *Staphylococcus aureus*
- ☐ *Streptococcus pneumoniae*
- ☐ *Vibrio cholera*
- ☐ Total coliform bacteria
- ☐ Others
- ☐ I don't know/I prefer not to answer

5.5.3.2 Please specify 'other':

100 character(s) maximum

* 5.5.3.3 Which of the following antibiotic-resistant groups of bacterial human pathogens from the WHO Bacterial Priority Pathogens list 2024 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR?

Maximum 15 selection(s)

- ☐ *Acinetobacter baumannii* carbapenem-resistant
- ☐ Enterobacterales third-generation cephalosporin-resistant
- ☐ Enterobacterales carbapenem-resistant
- ☐ *Enterococcus faecium* vancomycin-resistant
- ☐ *Haemophilus influenzae* ampicillin-resistant

- ☐ *Mycobacterium tuberculosis* rifampicin-resistant
- ☐ *Neisseria gonorrhoeae* third-generation cephalosporin, and/or fluoroquinolone-resistant
- ☐ *Pseudomonas aeruginosa* carbapenem-resistant
- ☐ *Salmonella Typhi* fluoroquinolone-resistant
- ☐ Non-typhoidal Salmonella fluoroquinolone-resistant
- ☐ Shigella spp. fluoroquinolone-resistant
- ☐ *Staphylococcus aureus* methicillin-resistant Group A
- ☐ Streptococci macrolide-resistant
- ☐ *Streptococcus pneumoniae* macrolide-resistant Group B
- ☐ Streptococci penicillin-resistant
- ☐ Others
- ☐ I don't know / prefer not to answer

5.5.3.4 Please specify 'other':

100 character(s) maximum

* 5.5.3.5 Which of the following fungal human pathogens from the WHO fungal priority pathogens list 2022 would be the MOST FEASIBLE AND INFORMATIVE option for culture-based AMR surveillance?

Maximum 15 selection(s)

- ☐ *Aspergillus fumigatus*
- ☐ *Candida albicans*
- ☐ *Candida auris*
- ☐ *Candida parapsilosis*
- ☐ *Candida tropicalis*
- ☐ *Coccidioides spp*
- ☐ *Cryptococcus gattii*
- ☐ *Cryptococcus neoformans*
- ☐ *Eumycetoma* causative agents
- ☐ Fusarium spp
- ☐ Histoplasma spp
- ☐ *Lomentospora prolificans*
- ☐ Mucorales
- ☐ *Nakaseomyces glabrata (Candida glabrata)*
- ☐ Paracoccidioides spp
- ☐ *Pichia kudriavzeveii (Candida krusei)*
- ☐ Pneumocystis jirovecii
- ☐ Scedosporium spp
- ☐ *Talaromyces marneffei*
- ☐ Others
- ☐ I don't know /I prefer not to answer

5.5.3.6 Other:

100 character(s) maximum

Identifying and monitoring antibiotic resistant genes (ARGs)

According to NCBI's bacterial genomic data, the highest diversity of submitted AMR gene sequences corresponds to resistance to beta-lactams, followed by aminoglycosides, quinolones, glycopeptides, tetracyclines and macrolides, among others:

* 5.5.3.7 Which of the following antibiotic resistance classes should be prioritized for the purpose of AMR surveillance?

Maximum 15 selection(s)

- ☐ Aminoglycoside resistance
- ☐ Amphenicol resistance
- ☐ Carbapenem and monobactam resistance
- ☐ Cephalosporins (First- and second-generation) resistance
- ☐ Cephalosporins (Third- and/or fourth-generation) resistance
- ☐ Fluoroquinolone resistance
- ☐ Glycopeptide resistance
- ☐ Imidazole derivative resistance
- ☐ Lincosamide and Streptogramin resistance
- ☐ Macrolide resistance
- ☐ Penicillin resistance
- ☐ Polymyxin resistance
- ☐ Tetracycline resistance
- ☐ Trimethoprim/sulphonamide resistance
- ☐ Other
- ☐ I don't know /I prefer not to answer

5.5.3.8 Other:

100 character(s) maximum

5.5.3.9 For each of the indicator purposes shown in the table below, which antimicrobial resistance gene targets would be the best for qPCR-based AMR surveillance? Choose up to 10 genes for each category. You can use the genes shown in table 3 in the annex 1 or add other genes you might know.

	AMR proxy / anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

5.5.3.10 How would you allocate the following types of gene indicators across environmental compartments for AMR surveillance and risk assessment purposes?

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Surface water	☐	☐	☐	☐	☐
Ground water	☐	☐	☐	☐	☐
Wastewater	☐	☐	☐	☐	☐
Water reuse	☐	☐	☐	☐	☐
Sludge (WWTP)	☐	☐	☐	☐	☐
Manure	☐	☐	☐	☐	☐
Soil	☐	☐	☐	☐	☐

5.5.3.11 How would you allocate the following types of gene indicators by the following objectives of AMR surveillance?

The above used objectives were:
1) Provide information about patterns and trends in AMR (including identification of emission sources)
2) Support and inform risk analysis of AMR in the environment
3) Alert on emergence and evolution of AMR
4) Assess the effectiveness of interventions

	Anthropogenic pollution markers	Clinically relevant ARG for human health risk assessment	Clinically relevant ARG for animal health risk assessment	Clinically relevant ARG for environmental health risk assessment	Predictors of diversity and evolution (mobility markers)
Monitor trends of AMR	☐	☐	☐	☐	☐
Provide data for risk assessment	☐	☐	☐	☐	☐
Determine emerging AMR forms	☐	☐	☐	☐	☐
Evaluate interventions	☐	☐	☐	☐	☐

5.5.3.12 ***Identifying and monitoring levels of antibiotic and antibiotic residues***

Which of the following antimicrobial classes should be monitored for AMR risk assessment?

Antibiotics	Antibiotics *Maximum 15 selection(s)* ☐ Aminoglycosides ☐ Amphenicols ☐ Carbapenems and monobactams ☐ First- and second-generation cephalosporins ☐ Fluoroquinolones ☐ Glycopeptides ☐ Imidazole derivatives ☐ Lincosamides and Streptogramins ☐ Macrolides ☐ Other ☐ Penicillins ☐ Polymyxins ☐ Tetracyclines ☐ Third- and/or fourth-generation cephalosporins ☐ Trimethoprim/sulphonamides
Antimycotics	Antimycotics *Maximum 5 selection(s)* ☐ Azoles ☐ Amphotericin B ☐ Echinocandins ☐ Terbinafine ☐ Others
Disinfectants / preservatives	Disinfectants / preservatives ☐ Triclosan ☐ Quaternary ammonium compounds QACs ☐ Chlorhexidine ☐ Chlorine-releasing compounds ☐ Aldehyde-based compounds ☐ Alcohols ☐ Hydrogen peroxide ☐ Peracetic acid ☐ Weak organic acids ☐ Others

* 5.5.3.13 **Defining criteria for risk assessment of antibiotics and antibiotic residues**

Which of the following already established indicators should be used to assess the selective potency of antibiotics for environmental risk assessments?

- ☐ Lowest Observed Effect Concentration (LOEC)
- ☐ No Observed Effect Concentration (NOEC)
- ☐ Predicted No Effect Concentration (PNEC)
- ☐ Minimal Selective Concentration (MSC)

- [ ] Minimal Inhibitory Concentration (MIC)
- [ ] Others
- [ ] I don't know / prefer not to answer

5.5.3.14 Please specify 'other':

100 character(s) maximum

* 5.5.3.15 Which of the following indicators currently being researched could be used to help assess the selective potency of antibiotics for environmental risk assessments?

Maximum 3 selection(s)

- [ ] Minimal concentration inducing resistance mutations
- [ ] Minimal concentration increasing the rate of horizontal transfer of mobile resistance determinants
- [ ] Minimal increased persistence concentration
- [ ] Total bacterial community growth
- [ ] Selective ability of whole effluents
- [ ] Length of time exposure to antibiotics
- [ ] ARB/ARG diversity and abundance
- [ ] Additive, synergistic or antagonistic effects of antibiotics and other chemical mixtures
- [ ] Seasonal variations of antibiotics
- [ ] Others
- [ ] I don't know / prefer not to answer
- [ ] None of the above

5.5.3.16 Please specify 'other':

100 character(s) maximum

5.5.3.17 To which extent do you agree with the idea of using WHO's AWaRe and EMA'AMEG antibiotic prescription's prioritization schemes for selecting the antibiotics for surveillance and risk assessment of AMR levels?

5.5.3.18 ***Identifying and monitoring levels of AMR drivers***

In future, which of the following natural physico-chemical water parameters would be the MOST FEASIBLE AND INFORMATIVE metadata of AMR to monitor?

- [ ] Temperature
- [ ] Oxygenation conditions
- [ ] Salinity
- [ ] pH
- [ ] Nutrient conditions
- [ ] Hydrodynamic conditions
- [ ] Others
- [ ]

None of the above

5.5.3.19 What other physico-chemical parameters would be feasible and informative to collect?

100 character(s) maximum

Methodological aspects

* 5.5.3.20 In future, which of the following methods below would be the MOST FEASIBLE AND INFORMATIVE to monitor AMR?

Maximum 10 selection(s)

- Absolute quantification of ARGs measured by qPCR
- Relative abundance of ARGs measured by qPCR
- Absolute quantification of ARGs measured by ddPCR/dPCR
- Relative abundance of ARGs measured by ddPCR/dPCR
- High-throughput real-time PCR methods (relative abundance of gene only)
- Shot-gun metagenomics
- Target enrichment metagenomics (i.e. enrichment of AMR genes in shot-metagenomics libraries)
- Quantitiative isolate-based methods -phenotypic assays (such as ESBL-producing E. coli in Tricycle protocol)
- Quantitiative isolate-based methods in combination with whole genome sequencing of isolates
- Isolate-based methods - proportion of resistant bacteria among isolates of a given species
- A combination of culture-based, qPCR and metagenomics
- A combination of culture-based methods and qPCR
- A combination of qPCR and metagenomics
- A combination of culture-based methods and metagenomics
- Other
- I don't know /I prefer not to answer

5.5.3.21 Please specify 'other':

100 character(s) maximum

* 5.5.3.22 What should be the preferred unit reported for absolute quantitative-based assays ?

Maximum 2 selection(s)

- Cumulative sum of all quantified ARG (e.g., ARGs/L)
- Gene copies / CFU per L
- Gene copies / CFU per g of dry mass (for solids)
- Gene copies / CFU per g of wet mass (for solids)
- Gene copies / CFU per g of ashes (for solids)
- Other
- I don't know /I prefer not to answer

5.5.3.23 Please specify 'other':

50 character(s) maximum

* 5.5.3.24 What should be the preferred relative abundance normalization method for culture-based and PCR assays?

Maximum 2 selection(s)

- ☐ *16S* rRNA gene copy number
- ☐ total DNA
- ☐ a collection of single-copy genes
- ☐ *rpoB* gene
- ☐ for culturing: concentration of non-resistant *E. coli*
- ☐ None
- ☐ Other
- ☐ I don't know /I prefer not to answer

5.5.3.25 Please specify 'other':

50 character(s) maximum

* 5.5.3.26 What should be the preferred relative abundance normalization method for sequencing-based methods?

Maximum 2 selection(s)

- ☐ *16S* rRNA
- ☐ a collection of single copy genes
- ☐ *FPKM*
- ☐ *TPM*
- ☐ *rpoB* gene
- ☐ spike-ins of strains at known concentration
- ☐ None
- ☐ Other
- ☐ I don't know /I prefer not to answer

5.5.3.27 Please specify 'other':

50 character(s) maximum

5.5.3.28 Would there be fundamental changes to your answers on 'indicators' if you would consider the far future (10 years) instead of the next 5 years? If so, please explain:

300 character(s) maximum

5.6 General questions future surveillance

Methodological barriers for monitoring AMR

* 5.6.1 What are the most challenging technical barriers regarding culture-based methods for ARB/ARG?

Maximum 3 selection(s)

- ☐ Cost
- ☐ Skill/training/labor requirement
- ☐ Insufficient sensitivity
- ☐ High detection limit
- ☐ Insufficient quantitation
- ☐ The time-to-result
- ☐ Inhibition effects
- ☐ Lack of standardised methods
- ☐ Other
- ☐ I don't know /I prefer not to answer

5.6.2 Please specify 'other':

100 character(s) maximum

* 5.6.3 What are the most challenging technical barriers regarding PCR detection methods for ARB/ARG?

Maximum 3 selection(s)

- ☐ Cost
- ☐ Skill/training/labor requirement
- ☐ Insufficient sensitivity
- ☐ High detection limit
- ☐ Insufficient quantitation
- ☐ The time-to-result
- ☐ Inhibition effects
- ☐ Inability to identify host of gene and the bacterial viability
- ☐ The lack of highly characterized ARG and their contribution to AMR
- ☐ The limited capability to detect extracellular vs intracellular DNA
- ☐ Lack of DNA extraction and amplification method standardization across bacterial species
- ☐ Other
- ☐ I don't know /I prefer not to answer

5.6.4 Please specify 'other':

100 character(s) maximum

* 5.6.5 What are the most challenging technical barriers regarding sequencing methods for ARB/ARG?

Maximum 5 selection(s)

- ☐ Cost
- ☐ Skill/training/labor requirement
- ☐ Insufficient sensitivity

- [ ] High detection limit
- [ ] Insufficient quantitation
- [ ] The time-to-result
- [ ] Inhibition effects
- [ ] Inability to identify host of gene and the bacterial viability
- [ ] The lack of highly characterized ARG and their contribution to AMR
- [ ] Lack of standardization with bioinformatic pipelines
- [ ] The added complexity of plasmid sequencing analysis
- [ ] The limited availability of highly curated gene repositories and reference databases
- [ ] Other
- [ ] I don't know /I prefer not to answer

5.6.6 Please specify 'other':

100 character(s) maximum

Costs of AMR monitoring

* 5.6.7 What cost per sample would make inclusion of AMR surveillance a realistic option?
  - ( ) Less than 10 €
  - ( ) 10-25 €
  - ( ) 25-50 €
  - ( ) 50-100 €
  - ( ) 100-200 €
  - ( ) 200 € or more
  - ( ) The price is not relevant
  - ( ) I don't know /I prefer not to answer

5.6.8 Do you have any other considerations regarding possible costs for future environmental AMR surveillance?

500 character(s) maximum

* 5.6.9 When do you think your country would be ready to implement standard methods for environmental AMR monitoring?
  - ( ) Now
  - ( ) 3 years
  - ( ) 5 years
  - ( ) 10 years
  - ( ) More than 10 years
  - ( ) I don't know /I prefer not to answer

6 Satisfaction

6.1 To what extent are you satisfied with the comprehensiveness of this survey on environmental surveillance of AMR?

6.2 To what extent are you satisfied with the usefulness of this survey on environmental surveillance of AMR?

6.3 Is there anything else you would like to share regarding environmental surveillance?

500 character(s) maximum

7 Survey A: Mapping existing environmental surveillance systems

A 'surveillance system' is defined here as a coherent approach towards environmental surveillance in one or more environmental compartments that uses a set of common indicators in a common set of samples in one timeframe. This includes

- national, regional and local surveillance systems,
- surveillance conducted regularly and repeatedly, but also surveillance executed only once in time (surveillance pilots),
- surveillance executed by governmental agencies, but also surveillance executed by research institutes.

7.1 Do you - or did you previously - have a surveillance system in place for antimicrobial resistance or other general pollutants that are related to AMR (such as antibiotics, or fungicides) in an environmental compartment?

- Yes (if you can describe one or more of them, please use the web link to the survey about existing environmental surveillance systems)
- No

8 References

- Krista Liguori et al, *Antimicrobial Resistance Monitoring of Water Environments: A Framework for Standardized Methods and Quality Control*, ACS Publications, 2022.
- Benedetti Guido, et al. *A survey of the representativeness and usefulness of wastewater-based surveillance systems in 10 countries across Europe in 2023*. Euro Surveill. 2024.
- Paracchini, V., Petrillo, M., Arcot Rajashekar, A. et al. *EU surveys insights: analytical tools, future directions, and the essential requirement for reference materials in wastewater monitoring of SARS-CoV-2, antimicrobial resistance and beyond*. Hum Genomics 18, 72 (2024)

Thank you so much for your contributions. If you have any questions, please contact your national contact point.

Please visit the website of EU-JAMRAI to learn more about the full project.

On behalf of the full team of EU-JAMRAI 2 work package 8.3 - Roosmarijn Luiken, Luis Lucena, Thibault Stalder, Christophe Dagot and Heike Schmitt.