VKM Report 2022: 28

Surveillance of antimicrobial resistance in the environment

Scientific Opinion of the Panel on Microbial Ecology

Norwegian Scientific Committee for Food and Environment

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft this opinion. The project group consisted of four VKM members, one VKM staff member, and three external experts. Two referees commented on and reviewed the draft opinion. The Committee, by the Panel on Microbial Ecology, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Microbial Ecology.

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Contents

Executive summary

This self-task report examines the basis for the establishment of a Norwegian surveillance programme for antimicrobial resistance (AMR) in the environment. The literature-based report summarizes evidence, identifies main knowledge gaps, and explores the rationale and relevance of a systematic approach to monitoring AMR in the environment.

Many countries with well-developed public health systems have established AMR surveillance programmes in clinical settings. The environmental dimension of resistance, however, lacks such standardized approaches, tools, and methodological frameworks. Our current understanding of environmental resistance is also severely limited by the complexity of the sites and processes involved. Most environmental studies are point prevalence-based snapshots of fragmented samples. Many samples taken from environmental sites may also be heavily influences by anthropogenic sources, for instance through dispersed wastewater and agricultural run-off. The scientific community has, in the context of the One Health paradigm, pointed out the many current knowledge gaps in our understanding of the environmental pillar of resistance. There is a lack of scientific understanding of how AMR in the environment carries a risk or hazard to human health – a gap, which needs future research. Moreover, there is limited understanding of the temporal and spatial separation between antimicrobial exposure and when and where resistance emerge. Therefore, many international initiatives have emerged to address the role of the environment in AMR development and to explore how the environment can be better recognized in surveillance programmes.

In this report, we assessed the basis for a systematic approach to environmental surveillance based on the One Health paradigm. The assessment took into account the methodology used in established surveillance programmes; the types, definitions, and descriptors of resistance; the experimental approaches used to determine resistance in the environment; important interfaces where genes, microbes, and antimicrobials originating from various environments meet; and current international initiatives for environmental surveillance (e.g. WHO, FAO, UNEP, EU). The key uncertainties and knowledge gaps encountered in the process are also presented along with conclusions and recommendations.

Several key findings emerged from our work. This includes the observation that calls for surveillance are not uniform nor straightforward to implement. Moreover, we find that the rationale for environmental surveillance differs from that driving clinical surveillance of AMR, and the methodology, tools and techniques used to determine environmental resistance need to be standardized. It is also clear that environmental populations of bacteria are considerably more diverse than the set of bacterial pathogens known to cause disease in humans and animals. These large differences in bacterial diversity and their heterogeneous environments exclude direct adoption of clinical sampling approaches to environmental settings. A new set of descriptors must therefore be developed and harmonized at the international level. These should accurately present the resistome of the overall microbial community rather than resistance traits present in single isolates.

An important part of the assessment outcome was the identified need for each surveillance effort to establish the rationale early and clearly for surveillance and the specific knowledge gaps addressed. Most of the expressed rationales of environmental surveillance are based on the need to strengthen a generally weak knowledge base that, at a later but unspecified stage, can be used in combination with clinical surveillance data to inform policy and interventions. It is important to recognize that rational environmental sampling will in most cases differ from that in clinical context due to a shift in focus from well-characterized species of pathogenic bacteria with known resistance profiles, clinical breakpoints, disease transmission pathways, and standardized protocols to a much broader set of (non-pathogenic) microbes present in diverse and unresolved microbial communities and

resistomes. Resistomes that in most cases will be a combination of resistance that is naturally present and resistance that originate from human activities.

The premises that must be met for the successful establishment of a "NORM-ECO" surveillance programme include that the rationale for surveillance is clearly defined, that the specific knowledge gaps addressed are clearly stated, that the surveillance effort is transparent in its description of uncertainty and risk, that the surveillance effort is based on that the most risk-relevant targets/sites/conditions have been identified, that the surveillance effort is an integrated part of a One health framework, and that data are collected through standardized methods and reporting that also include context and variability.

We emphasize the need to draw on existing sampling efforts to reduce costs. There is an opportunity to initially narrow the bacterial diversity sampled to those well-characterized species also occurring in clinical/anthropogenic contexts, for instance, the gut bacterium *Escherichia coli*. In all cases, a new environmental surveillance approach should be based on careful consideration of achievable goals, contextualization with other surveillance efforts, prioritization of the most relevant target populations, sites, and time intervals, and refinement of sampling methodology and standardization of data formats for comparative purposes.

We conclude that there is a scientific rationale, methodological opportunity, and broad support for the establishment of a "NORM-ECO" programme in Norway. The programme should focus on the effects of anthropogenic practices on the environment and should build on current international initiatives. Further work is needed to develop a technical framework that can guide environmental surveillance. Establishing a standing One Health-oriented scientific work group would be useful to bridge the human, animal, and environmental dimensions of resistance and to identify the most pressing knowledge gaps, the most AMR-relevant pollutants, and the most risk-relevant scenarios in Norway.

The scientific community should therefore work further to establish the biological basis of a NORM-ECO approach in Norway. This includes prioritizing among designs and key analytes to ensure that the programme contributes with longitudinal data collected in a systematic manner, is resource effective, builds on other environmental and clinical sampling programmes, is developed in a step-wise, adaptable, and scalable manner, and benefits from rapid advances in technology and data processing capacity.

Key words: Antimicrobial resistance, Environment, Norwegian Scientific Committee for Food and Environment, VKM, Surveillance, Monitoring

Sammendrag på norsk

Dette selvinitierte arbeidet undersøker det faglige grunnlaget for å etablere et norsk overvåkingsprogram for antimikrobiell resistens (AMR) i miljøet. Rapporten oppsummerer kunnskapsgrunnlaget, identifiserer viktige kunnskapshull og vurderer relevansen av en systematisk tilnærming til overvåking av AMR i miljøet.

De fleste industrialiserte land med velutviklede folkehelsesystemer har etablert overvåkingsprogrammer for antimikrobiell resistens i kliniske sammenhenger. Miljødimensjonen av AMR mangler imidlertid i slike standardiserte tilnærminger, verktøy og metodologiske rammer. Vår nåværende forståelse av miljøresistens er også sterkt begrenset av mangel på biologisk kunnskap for aktuelle miljøer. De fleste miljøstudier presenterer øyeblikksbilder og er basert på lite systematisk prøvetaking. Mange miljølokaliteter kan også være påvirket av menneskeskapte kilder, for eksempel gjennom avløpsvann og jordbruksavrenning. Miljøprøver vil derfor ofte være sammensatt av AMR fra ulike kilder, og det kan være vanskelig å skille disse kildene fra hverandre. I sammenheng med Én helse-paradigmet har forskningsmiljøer påpekt at det er mange kunnskapshull om AMR i miljøet, og mangel på vitenskapelig forståelse av hvordan - og om - AMR i miljøet medfører risiko for menneskers helse. Det er også begrenset kunnskap om den tidsmessige og romlige adskillelsen mellom antimikrobiell eksponering, og når og hvor resistens oppstår. For tiden tas det mange internasjonale initiativ for å peke på miljøets rolle i utvikling av AMR, og for å utforske hvordan miljøet kan inkluderes bedre i overvåkningsprogrammer.

VKM vurderte grunnlaget for en systematisk tilnærming til miljøovervåking basert på Én helse-paradigmet. Vurderingen la til grunn metodikken som brukes i etablerte overvåkningsprogrammer; typer, definisjoner og beskrivelser av resistens; de eksperimentelle tilnærmingene som brukes til å bestemme resistens i miljøet; viktige grensesnitt der gener, mikrober og antimikrobielle stoffer som stammer fra ulike miljøer møtes; og gjeldende internasjonale initiativer for miljøovervåking (f.eks. WHO, FAO, UNEP, EU). De viktigste usikkerhetene og kunnskapshullene presenteres sammen med konklusjoner.

Arbeidet ga flere funn. Vi fant blant annet at pågående forskning og initiativer knyttet til overvåkning av AMR i miljøet ikke er ensartet, eller metodisk enkel å gjennomføre. Videre fant vi at begrunnelsen for miljøovervåking skiller seg fra den som ligger til grunn for klinisk overvåking av AMR, og at metodikken, verktøyene og teknikkene som brukes til å bestemme miljøresistens må standardiseres. Det er også klart at miljøpopulasjoner av bakterier er betydelig mer varierte enn bakterielle patogener, som er kjent for å forårsake sykdom hos mennesker og dyr. Disse store forskjellene i bakteriemangfold og grad av heterogene miljøer utelukker at kliniske prøvetakingsmetoder kan brukes direkte på miljøprøver. Dersom AMR i miljøet skal overvåkes, påpeker VKM at et nytt sett med metoder og beskrivelser må utvikles og harmoniseres på internasjonalt nivå. Disse bør kunne beskrive den samlede forekomsten av resistens (resistomet) til det aktuelle mikrobielle samfunnet, i stedet for resistensegenskaper i enkeltisolater.

Faggruppe for mikrobiell økologi fremhever at det er nødvendig å identifisere kunnskapsbehovet som ligger bak hvert enkelt overvåkingsarbeid, på et tidlig tidspunkt. De fleste begrunnelser for miljøovervåking er basert på behovet for å styrke et generelt svakt kunnskapsgrunnlag, som på et senere stadium kan brukes i kombinasjon med kliniske overvåkingsdata til bruk for retningslinjer og intervensjoner. Det er viktig å erkjenne at prøvetaking i miljøet ofte vil avvike fra klinisk prøvetaking. Årsaken er at man må skifte fokus fra kjente arter av patogene bakterier med klarlagte resistensprofiler, kliniske brytningspunkter, sykdomsoverføringsveier og standardiserte protokoller, til et bredere sett med ikke-patogene mikrober som finnes i forskjellige og ikke kartlagte mikrobielle samfunn og resistomer. Slike resistomer vil i de fleste tilfeller være en kombinasjon av resistens som finnes naturlig og resistens som stammer fra menneskelige aktiviteter.

Dersom det skal etableres et overvåkingsprogram for AMR i miljøet, finner faggruppen at følgende premisser må være på plass: Den faglige begrunnelsen for overvåking må være klart definert. Kunnskapshull må være tydelig angitt. Overvåkingen må beskrive usikkerhet og risiko åpent. De mest relevante analyttene, prøvetakingssted og betingelser må være identifisert. Overvåkingen må være en integrert del av Én helse-rammeverket. Dataene må samles inn gjennom standardiserte metoder og rapportering som også inkluderer kontekst og variasjon.

VKM fremhever muligheten for å bruke eksisterende prøvetakingsarbeid i fremtidige vitenskapelige studier. I startfasen er det mulig å begrense bakteriemangfoldet som analyseres til de godt karakteriserte artene som også forekommer i kliniske / menneskeskapte sammenhenger, for eksempel tarmbakterien *Escherichia coli.* I alle tilfeller bør en ny miljøovervåkingstilnærming baseres på nøye vurdering av oppnåelige mål, kontekstualisering med andre overvåkningstiltak, prioritering av de mest relevante målpopulasjonene, stedene og tidsintervallene, og raffinering av prøvetakingsmetodikk og standardisering av dataformater for komparative formål.

VKMs faggruppe for mikrobiell økologi konkluderer med at det er tydelig behov for mer kunnskap om miljøresistens og mulig risiko. Det er metoder tilgjengelig som kan benyttes ved en eventuell etablering av et «NORM-ECO"-type -program i Norge, som ser på effekter av menneskelig aktivitet på miljøet og som samspiller med og understøtter internasjonale initiativer. Faggruppen oppfordrer til videre arbeid med utvikling av det vitenskapelige rammeverket for miljøovervåking. For eksempel kan det være nyttig å etablere en fast helseorientert faglig arbeidsgruppe for å bygge bro over resistensutfordringer som krysser menneskelige-, dyre- og miljødimensjoner og identifiserer de mest presserende kunnskapshullene, de mest AMR-relevante miljø agens, og de mest risikorelevante scenariene i Norge.

Faggruppen finner at grunnlaget for en NORM-ECO-tilnærming i Norge kan utvikles videre. Det inkluderer forståelsen av og prioritering mellom programdesign og sentrale analytter for å sikre at data samles inn på en systematisk måte, er ressurseffektiv, bygger på andre nasjonale og internasjonale miljømessige og kliniske prøvetakingsprogrammer, utvikles på en trinnvis, tilpasningsdyktig og skalerbar måte, og drar nytte av raske fremskritt innen teknologi og databehandlingskapasitet.

Abbreviations

AMR	Antimicrobial resistance
ARB	Antimicrobial resistant bacteria
ARG	Antimicrobial resistance gene
AGISAR	The WHO Advisory Group on Integrated Surveillance of antimicrobial Resistance
COL-R	Colistin resistance
CORNELIA	Antimi**cr**o**o**bial **R**esista**n**c**e** in One Hea**l**th **I**nterf**a**ces
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological cut-off
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESBL	Extended-spectrum beta-lactamase
ESC	*E. coli* resistant to extended-spectrum cephalosporins
ESVAC	The European Surveillance of Veterinary Antimicrobial Consumption
FAO	Food and Agricultural Organisation of the United Nations
GLASS	Global Antimicrobial Resistance and Use Surveillance System
HGT	Horizontal gene transfer
JPIAMR	The Joint Programming Initiative on Antimicrobial resistance
LA-MRSA	Livestock methicillin-resistant *Staphylococcus aureus*
LUCAS	Land Use/Cover Area Frame Statistical Survey
MALDI-TOF MS	Matrix-assisted laser desorption-ionization time-of-flight – mass spectrometry
MBC	Minimum bactericidal concentration
MDR	Multidrug resistant/Multidrug resistance

MIC	Minimum inhibitory concentration
MAGs	Metagenome assembled genomes
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSP	Methicillin resistant *Staphylococcus pseudintermedius*
MSC	Minimum selective concentrations
NEA	Norwegian Environment Agency
NIBIO	Norwegian Institute of Bioeconomy Research
NIVA	Norwegian Institute for Water Research
NVI	Norwegian Veterinary Institute
NORM	The Norwegian monitoring programme for AMR in human pathogens
NORM-VET	The Norwegian monitoring programme for AMR in animal pathogens
PCR	Polymerase chain reaction
PTM	Potentially toxic metals
QREC	Quinolone-resistant *E. coli*
UNEP	UN Environment Programme
VKM	Norwegian Scientific Committee for Food and Environment
VMP	Veterinary Medicinal Products
VRE	Vancomycin-resistant Enterococci
WGS	Whole-genome sequencing
WHO	World Health Organization
WWT	Wastewater treatment
WWTPs	Wastewater treatment plants

Glossary

Acquired resistance: Resistance to a particular antimicrobial agent to which the microorganism was previously susceptible. The change in resistance level is the result of genetic changes in a microorganism due to mutation(s), the acquisition of foreign genetic material, or a combination of both mechanisms.

Agricultural run-off: Run-off water from agricultural land/operations that can contain manure, pesticides, fertilizers etc.

Antibiotics: This traditionally refers to natural organic compounds produced by microorganisms that act in low concentrations against other microbial species, mostly bacteria. Today "antibiotics" also includes synthetic (chemotherapeutic) and semi-synthetic compounds (chemically modified antibiotics) with similar effects.

Anti-fungal agents: An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host.

Antimicrobial agents: A general term for the drugs (antibiotics), chemicals, or other substances that either kill or inhibit the growth of microbes. The concept of antimicrobials applies to antibiotics, disinfectants, preservatives, sanitizing agents, and biocidal products in general.

Antimicrobial resistance (AMR): A property of microorganisms that confers the capacity to inactivate or exclude antimicrobials, or a mechanism that blocks the inhibitory or killing effects of antimicrobials.

ATC: The Anatomical Therapeutic Chemical classification is an internationally accepted classification system for medicines that is maintained by the World Health Organisation (WHO).

Bacteriostatic effect: The agent prevents the growth of bacteria.

Biocides: Active substances and preparations containing one or more substances intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.

Biofilm: Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid/liquid) and are typically surrounded by an extracellular polymeric slime matrix. Flocs are suspended aggregates of microorganisms surrounded by an extracellular polymeric slime matrix that is formed in liquid suspension.

Clinical breakpoint: The breakpoint is defined as the MIC of antibiotic, above which the antibiotic on standard dose will result in sufficient concentration and sufficient duration to inhibit growth/eradicate the bacteria at the site of infection.

Conjugation: Transfer of genetic material between different bacterial cells by direct cell-to-cell contact.

Co-resistance: Resistance occurring when the genes specifying different resistant phenotypes are genetically linked or present in the same cell, for example, by being located together on one or more mobile genetic elements (e.g., a plasmid, transposon, or integron).

Cross-resistance: Resistance occurring when the same or similar mechanism(s) of resistance apply to different antimicrobials.

Disinfectants: Chemical substances that are designed to kill or inactivate microorganisms on non-living objects.

Epidemiological cut-off (ECOFF): These values are based on microbiological studies comparing AMR levels in new isolates to those observed in wild type populations and do not necessarily indicate whether a drug will be clinically active because they do not take into account what happens to an antibiotic within the body (pharmacokinetics). ECOFF values are not the same as clinical breakpoint concentrations.

Environmental compartment: The environmental compartments are, for example, water, soil, sediment, biofilms on various surfaces, wild animals, etc. Only a minor fraction of an environmental compartment is analysed (e.g., for fish, the liver might be analysed, and for water or soil it might be filtered/sieved water/soil that is analysed).

Environmental matrix: The type of environmental sample source such as different types of water, soil, or air.

Environmental resistance: resistance observed in bacterial communities sampled from the environment.

Fertiliser: Any material of natural or synthetic origin (other than liming materials) that is applied to soil or to plant tissues to supply one or more plant nutrients essential to the growth of plants.

Indicator bacteria: Bacteria that are used to measure the hygienic conditions of food, water, processing environments, etc. Indicator bacteria are not usually pathogenic, but their presence indicates that the product or environment tested may be contaminated with pathogenic bacteria, often originating from the same reservoirs as the indicator organisms.

Integron: Integrons are DNA elements with open reading frames (gene cassettes) that are expressed from a shared promoter. The level of expression depends on the distance from the promoter

Isolate: Bacteria that are recovered from a sample and grown in pure culture in the laboratory.

Manure: animal manure consists of organic matter from animal feces and plant-based composting material. Can be in liquid or solid form.

Microbiota: Collective term for a microbial community (i.e., any types of microorganism) that may be found within a given environment.

Minimum inhibitory concentration (MIC): The lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions.

Monitoring: the process of systematic observation over time. Monitoring for the specific purpose of guiding public health policy development and interventions is here defined as surveillance (see below).

Potentially toxic metals (PTMs): Naturally occurring elements that usually have a high atomic weight and a density at least 5 times greater than that of water.

Resistome: The collection of genes that contribute to antimicrobial resistance in microbial cell, population, or community.

Selection (bacteria): A process by which some bacterial species or strains in a population are selected for due to having a specific growth or survival advantage over other microorganisms. Antibacterial substances may provide a more resistant sub-population with such an advantage, enabling them to increase their relative prevalence.

Sewage: Describes the type of wastewater that is produced by a group of people in settlements of any size. It contains the effluents from households, small commercial or industrial entities, and, most often, surface runoff. See also "Wastewater". Often the term "wastewater" is used when sewage is meant. More precisely, "urban wastewater", "municipal wastewater", or "urban effluent" should be used instead.

Sludge: During municipal sewage treatment, biosolids (or sludges) are produced. Biosolids are product of physical (primary treatment), biological (activated sludge), and (physicochemical precipitation of suspended solids by) chemical treatment processes.

Surveillance: The process of continuous, systematic collection, analysis, and interpretation of data for the purpose of guiding public policy development and practice.

Susceptibility: Describes the vulnerability of a target microorganisms to an antimicrobial agent.

Transduction: The transfer of genetic material from one bacterial cell to another via bacteriophages (viruses that infect bacteria).

Transformation: Direct uptake from the environment of fragments of naked DNA and their incorporation into the bacterial cell's own genome.

Transposon: A segment of DNA that can move into a new position within the same or another chromosome or plasmid. Also called a jumping gene.

Wastewater: Any water that is discharged having been affected by human activities. This might be wastewater from households, wastewater from industry, or wastewater from point sources such as hospitals. Often the term "wastewater" is used as a synonym for sewage. See also "Sewage" and agricultural run-off

1. Introduction

Antimicrobial resistance (AMR) can be described as a microbe's ability to withstand the action of an antimicrobial agent (e.g. antibacterial agents, anti-fungal agents, potential toxic metals (PTMs), and disinfectants). AMR causes increased morbidity and mortality due to the lack of effectiveness of antimicrobials in both humans and in farmed animals and is a rapidly growing problem throughout the world.

The One Health paradigm points out that human and animal health is tightly linked to each other and to the health of the environment. AMR is routinely found also in the environment, such as in bacterial populations present in soil, freshwater, oceans, sedimentary deposits, and wild animals. Moreover, antimicrobial resistant bacteria (ARB) are continually released into the open environment from sources such as agricultural run-off, manure, sewage, and wastewater treatment plants (WWTPs). At the quantitative level, environmental populations of bacteria are expected to carry major parts of the global pool of resistance traits.

The dynamics of AMR are not yet fully understood within a One Health perspective (Figure 1). A key question is to what extent resistance genes from pathogenic bacteria in humans and animals that reach the environment, e.g. through sewage and manure, may have cyclic patterns and may affect treatment of pathogens in the future. Identical and similar resistance traits to those found in pathogenic human and veterinary isolates are sometimes found in environmental settings, but the events and mechanisms behind the shared ancestry are often undetermined. Moreover, novel resistance traits can be observed in environmental samples of bacteria. Thus, several pathways exist for clinical resistance to emerge from the environment or to relocate back to clinically relevant bacterial populations. Our understanding of the environmental reservoirs of resistance, transfer potential, and relevant pathways in the environment is currently limited by the complexity of the processes involved. The transfer and dissemination pathways of resistance are diverse and multi-dimensional in nature. Further complexity is added by the distribution of processes in space and time, a certain level of randomness, and a rational skewing in data collection to bacterial populations of direct clinical relevance.

Monitoring programmes for the development of resistance to antimicrobial agents have been developed for two of the three pillars of the One Health paradigm, namely human and veterinary health. In Norway these are the NORM and the NORM-VET programmes, respectively. The rationale and methodology for surveillance of resistance in disease-causing microbes in humans and animals have been worked out and refined at the international level for several decades. The insights gained from the repeated and targeted observations of the dynamics of resistance development in clinical/veterinary pathogens are used to guide treatment choices and contribute to optimal drug use in humans as well as in animal husbandry.

Monitoring programmes for resistance development to antimicrobial agents have not been developed to the same extent for the 3rd pillar of the One Health paradigm: environmental health. The current lack of systematic and longitudinal monitoring of environmental compartments can be explained by several factors: i) the rationale and methods for surveillance of microbes in environmental settings would necessarily differ as these are not intentionally treated with antimicrobials and immediate effects are not expected, ii) only a minor subset of the vast pool of microbial species and communities present in diverse environments cause disease in human and animals and are hence of immediate concern, iii) observed changes in resistance patterns in environmental microbes will not immediately lead to treatment failures in clinical settings or therapy regimes. Thus, causality, as well as the link between observation and the outcome of policy action (interventions) cannot be

immediately established. Because of this, there are today no established mechanisms or policies for transfer of insights and knowledge or the resistance situation in one environmental context to another.

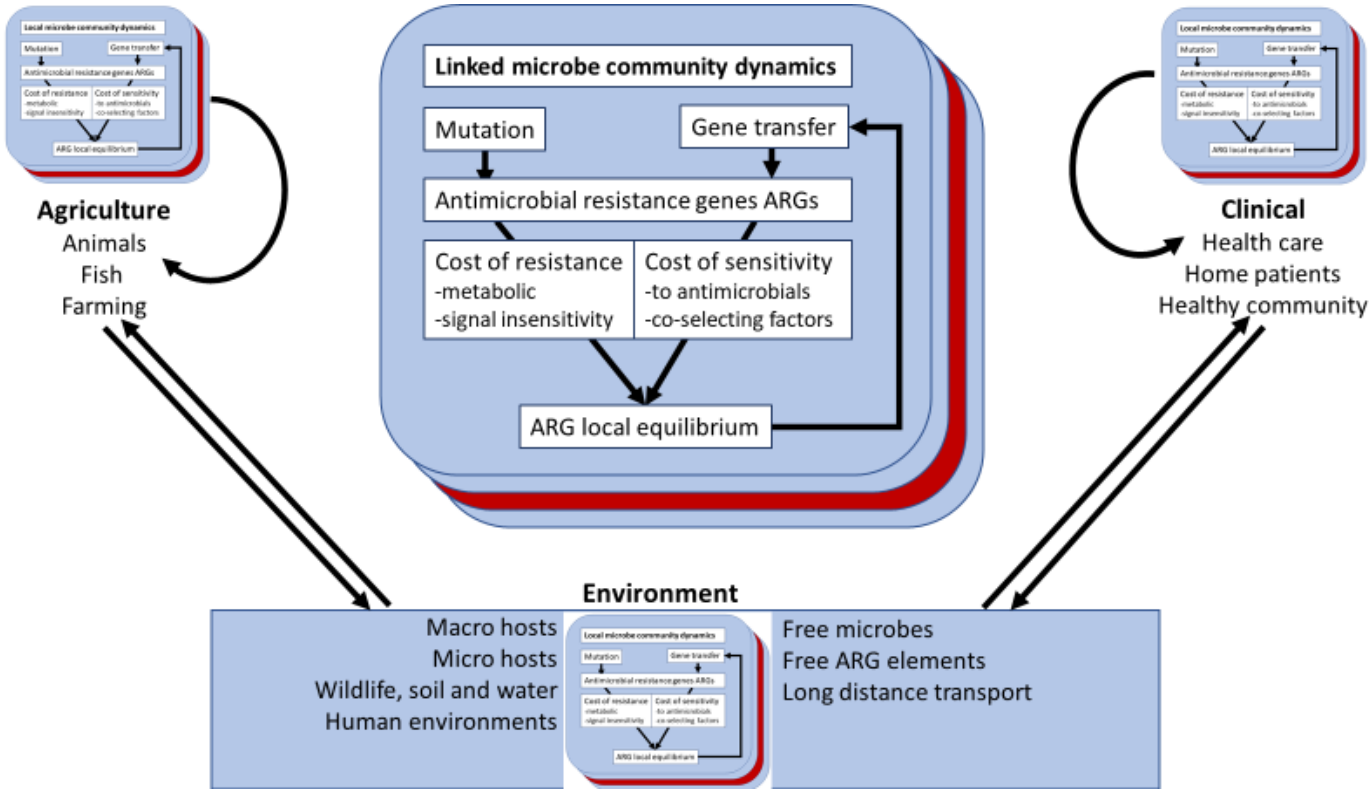


Figure 1 Schematic illustration of the dynamics of AMR in environmental, human, and agricultural compartments. The complexity and multi-layered dynamics within and between the three main compartments are shown in the middle of the figure. Each layer consists of multiple species and habitats, and the layers are linked on multiple scales. The red layers represent the pathogens that can be found embedded in each compartment as a part of the community in which AMR genes circulate (Figure by Kyrre Kausrud).

The One Health paradigm has emphasized the relations between human, animal, and environmental health (Box 1, see below). There is now a steady increase in initiatives addressing the role of the environment in AMR development and exploring how the environment can be better recognized in surveillance and control programmes, for example, in the environmental arm of the Global Antimicrobial Resistance and Use Surveillance System (GLASS) (https://www.who.int/initiatives/glass).

Recognizing such initiatives and the increasing scientific interest and emphasis of the environmental dimensions of antimicrobial resistance, the VKM panel on Microbial Ecology established a working group with a mandate that focused on exploring the factors determining the feasibility and usefulness of establishing a monitoring programme for environmental resistance in Norway.

In this report, we consider, at the general level, the current descriptors and approaches designed to understand resistance in the environment with the aim to understand their possible utility in longitudinal monitoring efforts. For instance, we examine the definition and description of resistance in the environment as well as the types of experimental design and methods available.

We then continue with an examination of current approaches to surveillance in order to understand how they can inform monitoring of resistance in the environment. This includes a view to the existing international arenas where approaches to resistance are negotiated and decided. Given the evidence base considered here, including a systematic review, we identify the types of knowledge gaps an environmental monitoring system might contribute to solve and the main associated contingencies. The evaluation builds on the One Health paradigm and a risk assessment approach including a hazard identification step.

Finally, we provide recommendations on if, and if so how, environmental surveillance might be developed and for what purpose. We focused on the development and spread of AMR in bacteria due to the use of antimicrobial agents. Development of resistance in archaea or eukaryotes such as fungi and parasites are not part of the assessment.

The two concepts of monitoring and surveillance are used throughout the report. These have various definitions according to the institutions defining them for the context they are applied (e.g. ECDC, EFSA, EMA). In this report we use both terms to describe the process of systematic observation of an environmental compartment over time (Figure 2).

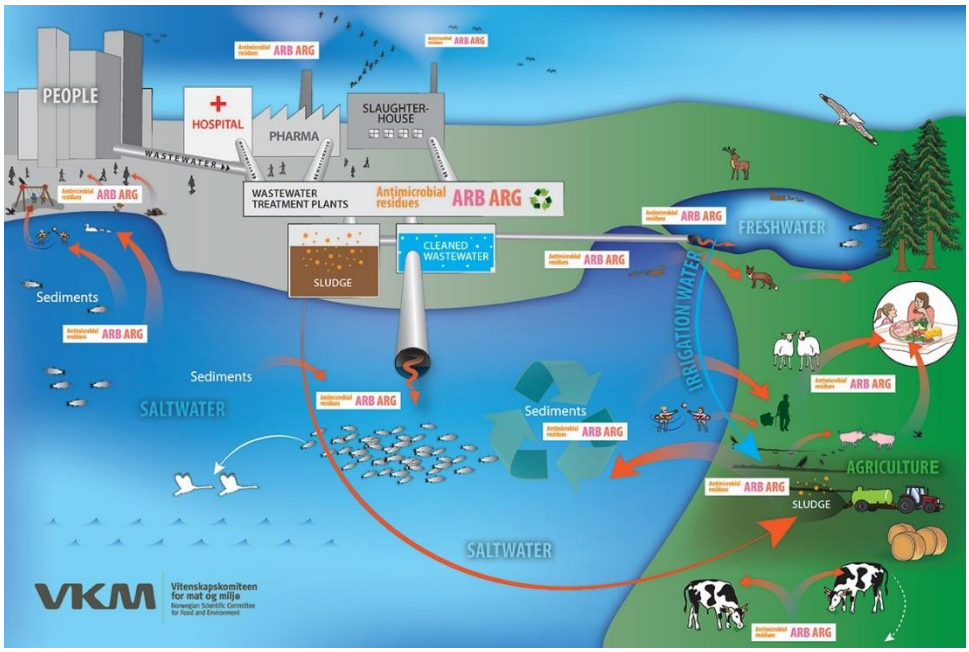


Figure 2. Informing One Health policy towards mitigating AMR through environmental monitoring. Surveillance informs policy aimed at mitigating the spread of AMR in human and agricultural systems and also serves to evaluate mitigation efforts to further develop policy and improve the efficacy of mitigation efforts (VKM 2020).

Box 1. The One Health paradigm

The One Health paradigm was launched at the beginning of our millennium as a response to the emergence and re-emergence of several zoonotic microorganisms being regarded as public health threats. In one of the key "One Health" founding articles, it was stated: "Physician and veterinarian comparative medicine research teams should be promoted and encouraged to study zoonotic agent-host interactions. These efforts would increase our understanding of how zoonoses expand their host range and would, ultimately, improve prevention and control strategies" (Kahn, 2006). The One Health idea was embraced by an array of research groups, institutions, and organizations, and it has since been developed and used in many different settings at different levels in our society. Cross-disciplinarity and intersectoral collaborations have always been a key element in the One Heath approach.

There has been some debate on how to define "One Health", and recently the WHO, OIE, FAO, and UNEP approved to the following definition: "One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, acting on climate change, and contributing to sustainable development." (https://www.who.int/news/item/01-12-2021-tripartite-and-unep-support-ohhlep-s-definition-of-one-health). This is a rather broad definition encompassing much more than AMR. AMR is, however, regarded as the quintessential aspect of One Health, as it exists in and affects all sectors (Essack, 2018). AMR in the environment is considered the most neglected part of One Health-AMR initiatives (Larsson and Flach, 2021).

Mandate

This is a self-task of the VKM´s Microbial Ecology panel that examines the basis for the possible establishment of a surveillance programme for environmental AMR. As mentioned above, monitoring for AMR is already established for human and domesticated animals in Norway. A comparable programme for the 3rd pillar of the One Health paradigm – environmental health – is currently lacking.

Terms of reference

1. Summarize the knowledge base supporting the definition and description of environmental AMR and propose a definition of acquired resistance in environmental bacteria based on established clinical definitions.

2. Evaluate available methods for resistance determination and the extent to which these are suitable for environmental samples, including the pros and cons of culture-based methods versus metagenome/whole genome-based methods (phenotype versus genotype).

3. Evaluate possibilities and limitations in sampling design and methods for sampling, sample material, and sample selection, including how this is carried out in today's NORM/NORM-VET approach.

4. Develop an overview of existing and planned approaches to environmental AMR monitoring nationally and internationally and assess how these can best be used in a potential NORM-ECO approach.

5. Base work on a comprehensive assessment of the knowledge base, identifying the main challenges, opportunities, and added value of establishing an ECO NORM monitoring programme.

6. After clarifying added value, suggest how further work in VKM can develop the programme, including identification of manageable selection of environment, samples, species, and methods that can provide a basis for standardization and time series.

Exclusions:

The following aspects have not been considered:

- Resistance in viruses, fungi, and other eukaryotes
- Chemical compounds (analytes) as resistance drivers other than pharmaceutically produced antimicrobials
- Environmental aspects of antimicrobial agents have only been considered to a limited extent

2. Assessment methodology and data sources

The literature-based assessment of environmental surveillance of AMR is based on a systematic review and evaluation of other published scientific literature, as well as reports and information available from various international initiatives (Fig. 3).

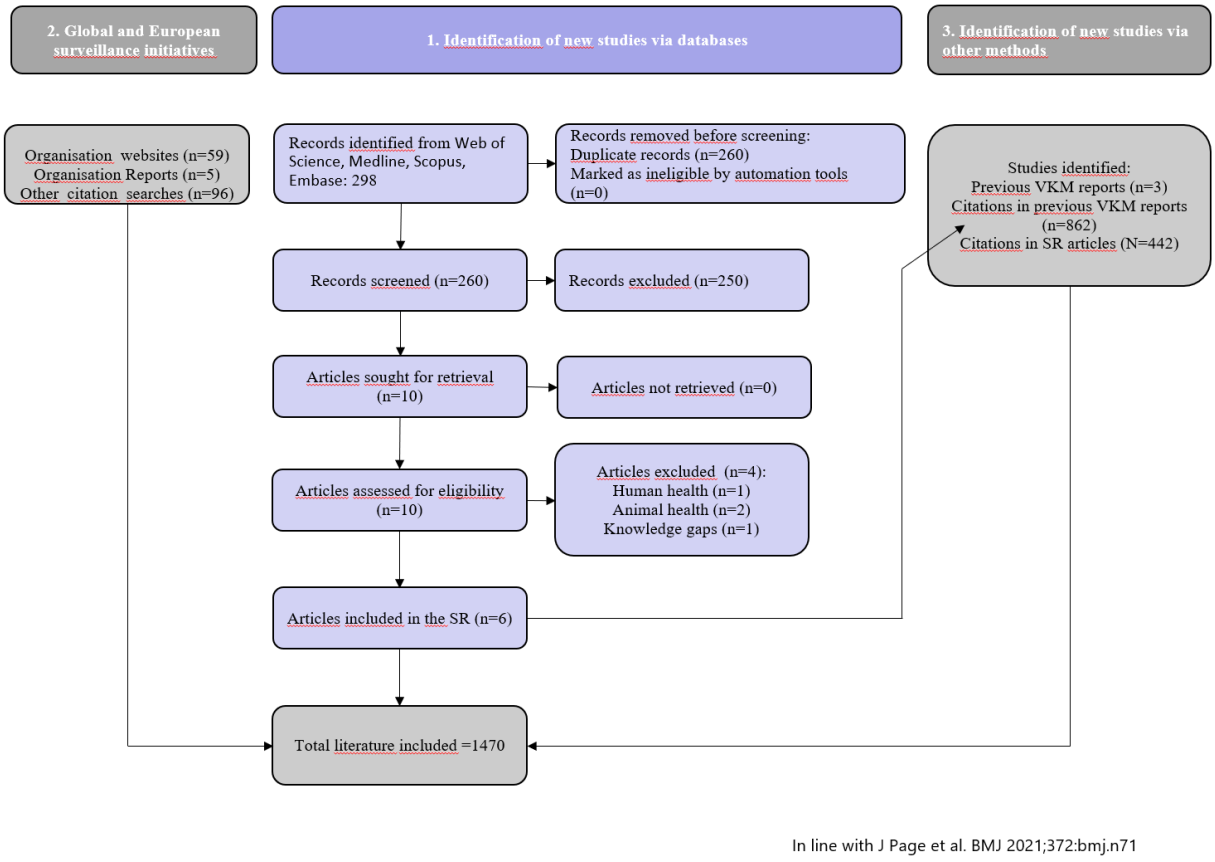


Figure 3. PRISMA 2020 Flow Diagram for Systematic Reviews and identification of other relevant knowledge used in this report.

Literature of relevance to the mandate was first identified through a systematic review (Figure 3, middle section). The systematic review identified six studies. Five of the six systematic reviews focused on AMR in water, namely untreated wastewater (n=3; (Chau et al., 2020) (Hamilton et al., 2020) (Hassoun-Kheir et al., 2020), groundwater (n=1; (Andrade et al., 2020)) and unspecified aquatic environments (n=1; (Coertze and Bezuidenhout, 2019)). None of the studies focused on soil or food-production environments specifically. One of the six reviews discussed how to define organizational and functional characteristics of One Health surveillance systems drawing on insight gained from the 41 different

surveillance systems considered (Bordier et al., 2020). In the Bordier review, AMR was the hazard in nine of them, dominated by surveillance across human and animal health and animal feed. None of the 41 systems considered included an environmental matrix.

For further description of the methods and outcomes of the systematic review, see Appendix 1.

Overall conclusions from our systematic review were:

- Limited scientific literature on environmental surveillance of AMR
- Single experimental studies were characterized by heterogeneity in samples, methods, and targets
- Comparative analyses between studies are difficult to perform due to lack of standardization
- Lack of an established consensus on which environmental compartments/sites should be examined and for what purpose
- Untreated wastewater could be a robust matrix to apply in AMR surveillance as an approximation for AMR levels in the general populations that are "sampled"
- Environmental matrices are a neglected or disregarded part of current AMR One Health surveillance systems

To cover the necessary attributes of a One Health surveillance system that addressed our broad mandate and included the environment, we extended the literature search (Figure 3) and performed our own synthesis. Additional peer-reviewed articles were therefore identified by members of the working group, as was relevant "grey" literature, such as national surveillance reports, VKM reports, and reports and other information (websites) from UN organizations, EU, and other science-based institutions (Figure 3). Identification of international initiatives on surveillance of AMR in the environment was based on expert's knowledge and web-search for relevant reports and information.

The resulting assessments and recommendations were based on conclusions drawn from the above-mentioned literature in combination with the professional expertise of the members of the working group.

3. Rationale for AMR surveillance in the environment

Most industrialized countries have established AMR surveillance programmes that are complemented with various tailored research agendas to address standing knowledge gaps. The environmental dimension of resistance lacks such standardized approaches, tools, and programmes. Hence, most of our current knowledge of environmental resistance is based on point prevalence-based studies of variable depth and coverage.

This discrepancy in the extent of systematic approaches and active surveillance efforts has several explanations. **First**, current surveillance efforts focus on well-characterized species of pathogenic bacteria with known resistance profiles and disease transmission pathways. **Second**, tools for isolation and the identification of single-species of bacteria and standardized protocols for resistance characterization are available. **Third**, observations of changing susceptibility patterns in the studied pathogens can be considered against clinical breakpoints and can inform best practice and clinical drug prescription guidelines. Hence, the collection of human and animal surveillance data directly informs clinical practice with the goal to prevent and control disease (CDC https://www.cdc.gov/csels/dsepd/ss1978/lesson5/section2.html). In contrast, for the environmental dimension of resistance, few, if any, of these data collection and usage conditions directly apply.

The rationale for surveillance of environmental populations of bacteria will thus differ from those developed for clinical pathogens – The perspectives from scientists, policy makers, stakeholders and authorities include:

- Build a better understanding of risks to human and animal health from/of:
    - the environment as a source for AMR development
    - mapping transfer/transmission pathways (genes, transferable genetic elements, or environmental pathogens), including directionality
    - the environment as intermediate sink/cyclic reservoir of resistance traits of clinical resistance (contaminated resistome)
- Develop a holistic understanding of ecosystem health – effects of anthropogenic activities/pollution across all three dimensions:
    - Identify biological effects of antimicrobial drugs and other selectors
    - inform on outcomes of interventions of drug usage in humans and animals, including zoonoses
    - contribute to big data-driven analysis and surveillance approaches at the international level
    - build a biobank and databank for research that facilitate comparable data analyses for assessing the relative impact to and from the environment
    - build intersectoral approaches for effective, multi-purpose surveillance
- Describe the environmental footprint of dispersed antimicrobials as pollutants:
    - causing accelerated evolution of ARM through unintentional exposure to drugs, genes, bacteria

    - identify other microbial community effects of antimicrobials in the environment
- Develop data-guided proxies (indirect measures)
    - for overall antimicrobial usage
    - AMR prevalence (including in humans, animals)
    - facilitate low-cost systems that can assist countries that need environmental surveillance due to a lack of resources for clinical surveillance
- Precautionary/proactive approaches to support the reduction of AMR and antimicrobial agents in the environment (based on the precautionary principle and acknowledging complexity, knowledge gaps, and uncertainty)
- Adhere to international obligations and policy directions as an outcome of the perspectives mentioned above (i.e., political and policy decisions, e.g., at the EU level)

The rationale behind environmental AMR is currently being explored at different levels. At the **international level**, several initiatives are now in development after deliberations at UN institutions such as the WHO, UNEP, FAO. In the EU, surveillance of AMR and antibiotics and their residues within the environment is part a response to the Strategy on Pharmaceuticals in the Environment, the revised EU AMR action plan, and the Green Deal initiative. These actions, as presented in further detail in chapter 4, represent a clear political direction at the international level. At the **national level**, a strengthening of the knowledge base is a prioritized effort in the National Strategy against AMR developed by the Norwegian Ministry of Health and Care Services in 2015 (National Strategy against Antibiotic Resistance 2015-2020. Publication number I-1164 E), where the following quote can be found: "The AMR problem is complex and there is still a lack of knowledge that must be addressed before we will have a holistic understanding of natural and man-made factors that prevent or promote the development of resistance".

The scientific community has certainly informed the above-mentioned initiatives. As a community they have also pointed out the many current knowledge gaps in our understanding of the environmental pillar of resistance. The role of anthropogenic influence on environmental resistance is an implicit research question in many research publications. Examples of concerted actions and calls for environmental surveillance of antimicrobials and antimicrobial resistance is presented in more detail in chapter 5.

4. AMR surveillance in the environment: International initiatives

4.1 Global level

The overarching goal of AMR surveillance as given by the **World Health Organization (WHO)** AMR Global Actions Plan and that forms the foundation of the WHO Global AMR Surveillance System is as follows:

> *'Surveillance is an essential tool to inform policies and infection prevention and control responses. Importantly, it is the cornerstone for assessing the spread of AMR and for informing and monitoring the impact of local, national and global strategies'*
>
> https://www.who.int/initiatives/glas ;
> https://apps.who.int/iris/rest/bitstreams/864486/retrieve

The UN Food and Agriculture Organization (FAO) states the following on AMR in the environment https://www.fao.org/documents/card/en/c/ca7442en/ (FAO 2020):

> "*Programmes and tools to systematically measure and record antimicrobial contamination and AMR bacteria in the environment at national levels are virtually absent. Environmental AMR surveillance systems need to be integrated and harmonized with surveillance in the human, animal, and food sectors to track the spread of antimicrobial residues, AMR organisms and ARGs to better assess the risks and priority areas for intervention"*

According to the July 2021 rolling action plan by the **Global Leaders Group on AMR**, the group will "Advocate for better understanding of environment pathways to the development and transmission of antimicrobial resistance" and states that a key activity under this priority is "integrated surveillance of antimicrobial discharge and determinants of AMR across all sectors" (Priorities of the Global Leaders Group on AMR for 2021-2022).

The Global Leaders Group on AMR performs an independent global advisory and advocacy role, with a mandate from the UN Secretary General to lead the global process of implementing the recommendation from the former UN Interagency Coordination Group on AMR, which concluded its mandate in 2019. Also, this group will be key to the UN high-level meeting on AMR scheduled for 2024. https://www.who.int/groups/one-health-global-leaders-group-on-antimicrobial-resistance

In March 2022, the Global Leaders group on AMR published a call to action for reducing antimicrobial discharges from food systems, manufacturing facilities, and human health systems into the environment. Alongside strengthened governance, improved discharge management, and research and development, the group calls for improved surveillance and data availability. https://www.amrleaders.org/news-and-events/news/item/02-03-2022-world-leaders-and-experts-call-for-action-to-protect-the-environment-from-antimicrobial-pollution

The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) recently developed the so-called Tricycle Project, now included into the Global Antimicrobial Resistance and Use Surveillance System (GLASS) as its 7th module – WHO One Health Survey (Tricycle protocol) (Table 1). The project has a strong work package on surveillance in the environment. The protocol (https://www.who.int/initiatives/glass/glass-modules-7) was piloted in nine countries representing all WHO regions and was recently made publicly available. Participation is at the national level in cooperation with relevant ministries. Extended spectrum beta-lactamase (ESBL)-producing *E. coli* was chosen as the target organism for feasibility reasons across resource settings. Furthermore, ESBL-*E. coli* are present in all domains of One Health and are also relatively easy to cultivate and identify. Alongside Enterococci, *E. coli* are commonly used as a marker of faecal pollution in water and thus are already well-known to the aquatic science community.

The WHO One Health Survey should not be mixed up with guidance on integrated surveillance of AMR in foodborne bacteria, developed by the same group a bit earlier. That guidance states that it applies One Health, but it focuses only on AMR and antimicrobial use in human and animal health and on the analyses across those two domains. https://apps.who.int/iris/bitstream/handle/10665/255747/9789241512411-eng.pdf?sequence=1&isAllowed=y

The WHO and FAO focus on AMR, and other organizations' concerted action within the framework of **Codex Alimentarius** recently led to the adoption of its very first Codex Alimentarius guideline on surveillance of AMR in food-production, including in food-production environments (Codex Alimentarius Guideline on Integrated Monitoring and Surveillance of Foodborne Antimicrobial Resistance, Appendix III). The potential political and economic impact of this guideline is major, as the World Trade Organization uses Codex documents when resolving trade disputes between countries. The Codex guideline nevertheless needs further operationalization, and the Codex Alimentarius Commission encouraged the WHO and FAO, in cooperation with the Codex Secretariate, to develop tools for implementation. The guideline also encourages the World Organization for Animal Health (OIE) and the International Plant Protection Convention (IPPC) to develop supporting texts that are in line with the guideline wording. Existing and future documents from the latter two are of similar importance to the World Trade Organization as those from Codex Alimentarius (Codex Alimentarius Commission 44 (2021) (point 80 Page 12, point 88 and 89).

During the 2022 **UN Environment Programme (UNEP)** assembly (https://apps.who.int/iris/rest/bitstreams/864486/retrieve), under the topics chemicals and pollution action, a summary for policy makers on the environmental dimensions of AMR was launched. Not surprisingly, UNEP mainly focused on the release of antimicrobial residues into

the environment, rather than the release of AMR pathogens. On the other hand, the report points to the need for documenting not only the release of antimicrobials, but also resistant microbes and their genetic material into the environment, as well as their impact on biodiversity. It is unclear, though, if the UNEP report will pave the way for including AMR-relevant pollution into the global monitoring that is under their custody, such as the Global Environment Monitoring System for freshwater (GEMS/Water, Appendix III).

4.2 European level

The European Union (EU) seeks to be recognized as a world leader in the fight against AMR. Several initiatives with a core focus on antimicrobial agents are currently running. In 2019, the European Commission adopted the **EU Strategic Approach to Pharmaceuticals in the Environment** https://ec.europa.eu/environment/water/water-dangersub/pharmaceuticals.htm in which antimicrobials are mentioned as pollutants from human and animal use as well as from the pharmaceutical industry. (https://ec.europa.eu/environment/water/water-dangersub/pdf/strategic_approach_pharmaceuticals_env.PDF).

The above-mentioned strategic approach has led to the inclusion of more antimicrobials in the updated Surface Water Watch List. The strategy has also pushed for the discussion to include antimicrobials when revising the EU Urban Wastewater Directive (Table 1).

For many years, the EU has surveyed land use and characteristics of the topsoil in the **Land Use/Cover Area frame statistical Survey (LUCAS;** Table 1 and Appendix III**)** https://esdac.jrc.ec.europa.eu/projects/lucas. Pharmaceutical concentrations and antimicrobial resistance genes have been analysed in the LUCAS 2018 soil survey, and has been proposed also for the 2022 samples (https://esdac.jrc.ec.europa.eu/public_path/u891/Proposal_paper_for_LUCAS2022_Soil-Final_xPUBSY.pdf)

Norway does not report to LUCAS, but it runs a similar surveillance within the Norwegian Agricultural Environmental Monitoring Programme (JOVA) led by the Norwegian Institute of Bioeconomy Research (NIBIO) and funded by the Norwegian Environment Agency https://www.nibio.no/tema/miljo/jord-og-vannovervaking-i-landbruket/metoder. To what extent probable changes in LUCAS will have an impact on JOVA is unclear. At the request of the Norwegian government, NIBIO recently assessed the needs for including surveillance of soil beyond just in agriculture and recommended a formalised participation in LUCAS soil (NIBIO rapport 2021;7(14) (In Norwegian). Jordsmonnet vi lever av – Forslag til system for dokumentasjon og rapportering av jordsmonnets tilsand og endring). https://nibio.brage.unit.no/nibio-xmlui/bitstream/handle/11250/2725540/NIBIO_RAPPORT_2021_7_14.pdf?sequence=2&isAllowed=y).

The report did not recommend including surveillance of pollutants yet, on the grounds of a lack of standardization. On the other hand, it points to the rapid development of molecular methods to assess biodiversity, although not mentioning AMR or antibiotic resistance genes (ARGs) specifically. There might be a placeholder for both antimicrobials as pollutants and AMR/ARGs as part of biodiversity evaluations (See Table 4, Page 3 in the report). Many European countries have started surveillance of SARS-CoV-2 in untreated wastewater during

the Covid-19 pandemic. These initiatives have been grouped under the so-called **EU Umbrella Sewer Study** (Appendix II, Table AII.5). Based on European Commission recommendations on covid 19 surveillance in waste water (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021H0472&from=EN), the Directorate General (DG) Environment considers regular monitoring of AMR under the Urban WAste Water Treatment Directive to be proposed later 2022 (https://ec.europa.eu/assets/sante/health/amr/docs/amr_20220126_co03_en.pdf). If this happens, it will probably have an impact on political decisions in Norway as well.

In Norway community WWTPs perform a range of routine analyses according to requirements defined in the Norwegian Regulation on Pollution Control, Part 4 Wastewater (https://lovdata.no/dokument/SF/forskrift/2004-06-01-931/kap11#kap11). It is noted that the current microbial analyses do not focus on the AMR characteristics of the samples.

4.3 Single country initiatives on surveillance of AMR in the environment

Both the WHO One Health Survey environmental arm and the global sewage study mentioned above sample wastewater for repeated snapshots of the level of both AMR and antimicrobial residues at the community level. When the COVID-19 pandemic struck, this community approach was also applied for surveying the SARS-CoV-2 virus in wastewater, with the goal to act as an early warning system and for detecting trends. Countries that were early in establishing wastewater epidemiological surveillance for the virus are currently evaluating ways to capitalize on the network, sampling logistics, and methods for SARS-CoV-2 surveillance and translate them into surveillance for AMR (ALW; pers. comm.). The Public Health Agency of Canada (PHAC) coordinates the Canadian effort to survey wastewater for the SARS-CoV-2 virus. The long-term goal is to use this system for other infectious diseases, including AMR (personal communication Michael Mulvey, PHAC). The survey has developed detailed guidance documents on sampling, transport, storage, analysis (quantitative PCR), quality control, etc., https://nccid.ca/wastewater-surveillance-for-covid-19/. Other public health institutes coordinate similar surveillance, such as New South Wales in Australia https://www.health.nsw.gov.au/Infectious/covid-19/Pages/sewage-surveillance.aspx, the Netherlands https://coronadashboard.government.nl/landelijk/rioolwater, and the US Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html). Several hundred sites are sampled and analyzed either by quantitative PCR or digital droplet PCR, and guidance on methods is provided (https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html) The US National Wastewater Surveillance System is expanding to AMR, and as of October 2020 it had sampled 50 WWTPs and performed analyses for several resistance gene groups, enabling rapid adaption to emerging concerns (personal communication) https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html .

Table 1. Focus areas of some international initiatives on surveillance of AMR and antimicrobial agents in the environment (see also tables in the Appendices).

	Focus	Detection method	Environmental matrix	Ripeness of initiative
WHO GLASS One Health Survey	ESBL-*E. coli* across human, animal, and environmental health	ARB (+WGS)	Untreated river water, Wastewater, Wet marked draining	**Tricycle Protocol**[a] Pilot in 9 countries. Reporting module in GLASS
WHO/FAO Codex Alimentarius	Guideline on Integrated Monitoring and Surveillance of **Foodborne AMR**[b]	ARB, WGS (*Salmonella, Campylobacter, E. coli,* Enterococci)[b]	"soil, water, litter and bedding, organic fertilizers, sewage or manure"[b]	Needs operationalizations and implementation tools[c]
EU Umbrella Sewer Study[d]	SARS-CoV-2, but planned for AMR and anti-microbials specified in the **EU 3rd Watch List**[e]	ARB, ARGs Mass spectrometry	Untreated waste-water	Proposal to include in revised **Urban Wastewater Treatment Directive**[f]
LUCAS soil monitoring[g]	Antimicrobials according to **EU 3rd Watch List**[c]	Mass spectrometry	Topsoil	Well established system with broad focus. Analyses of antimicrobials in implementation phase

[a]GLASS One Health Survey: Tricycle protocol: https://www.who.int/initiatives/glass/glass-modules-7

[b]Codex Alimentarius Guideline on Integrated Monitoring and Surveillance of Foodborne Antimicrobial Resistance, Appendix III: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/de/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-804-08%252FREPORT%252FFinalReport%252FREP21_AMR08e.pdf

[c]Codex Alimentarius Commission 44: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-701-44%252FDRAFT%2BREPORT%252FDraft%2BRep21_CAC%2Bwithout%2Bdiscussion%2BZilpaterol%2BItem%2B2%2Band%2Bitem%2B11.pdf

[d]EU Umbrella study on SARS-CoV-2 in sewage: https://ec.europa.eu/jrc/en/science-update/sars-cov-2-surveillance-employing-sewers-eu-umbrella-study-status-update

[e]Third Watch List under the Water Framework Directive: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32020D1161&from=EN

[f] Revision of EU Urban Waste Water Treatment Directive https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/12405-Revision-of-the-Urban-Wastewater-Treatment-Directive/public-consultation_en

[g]LUCAS soil monitoring: https://esdac.jrc.ec.europa.eu/projects/lucas

IPPC = International Plant Protection Convention, led by the Commission on Phytosanitary Measures, IPPC Secretariat at FAO.

5. AMR surveillance in the environment: calls from the scientific community

At the scientific community level, a call for closing the many knowledge gaps and for monitoring the levels of antimicrobial agents and AMR in the environment has been made. Some recent examples of both national and international initiatives are listed below.

Research networks and projects

The Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) facilitates coordinated research on AMR at the global level. The JPIAMR has 29 member countries and is supported by the EU (https://www.jpiamr.eu). Priority topics on the Strategic Research and Innovation Agenda include: *"Surveillance: Optimisation of surveillance systems to understand the drivers and burden of antimicrobial resistance in a One Health perspective"* and *"Environment: The role of the environment in the persistence, selection and spread of antimicrobial resistance".*

The Environmental dimensions of antimicrobial resistance (EDAR) biannual international conference series that started in 2012; https://www.gu.se/en/care/edar6-gothenburg-2022 offers a key knowledge sharing and research networking platform.

EDAR (https://www.gu.se/en/care) is also a multidisciplinary project funded by the Swedish Research Council for the period 2019–2024 and is led by Joakim Larsson at the University of Gothenburg. The project addresses several critical knowledge gaps related to the environmental dimensions of AMR, but it does not focus on surveillance as such:

> "Understand the origin and evolution of AMR, i.e. in what bacterial species and from what environments they likely were mobilized and transferred.
> Identify already mobilized resistance genes to last-resort antibiotics that have not (yet) been described in pathogens.
> Understand drivers and mechanisms of resistance evolution (selection, mobilization, transfer) in the environment.
> Provide an economic analysis of the costs and benefits of environmental interventions.
> Analyse incentives and counterincentives for such mitigations".

The University of Gothenburg also hosts the Centre for Antibiotic Resistance Research (CARe) https://www.gu.se/en/care/edar6-gothenburg-2022.

Establishing a Monitoring Baseline for Antimicrobial Resistance in Key environments (EMBARK) https://antimicrobialresistance.eu/ is funded by JPIAMR (Diagnostics and surveillance call 2019). The project is coordinated by Johan Bengtsson-Palme at the University of Gothenburg and has participants from Germany, France, Pakistan, and China.

EMBARK will produce a monitoring scheme that will be designed so that it can be used modularly depending on the available resources, leading to more efficient use of money and time and enabling environmental monitoring of resistance where it might be most needed, namely in low-income countries with poor hygiene standards.

EMBARK will:

1. "establish baseline ranges for background ARG abundances and diversity in different environments,
2. standardize different methods for monitoring ARGs and provide a means for making them comparable,
3. identify sets of priority target ARGs for monitoring,
4. develop methods to detect emerging resistance threats and thereby provide an early-warning system for resistance, and
5. suggest a monitoring scheme that can be used in a modular fashion depending on the available resources".

The global sewage study started as a part of the large COMPARE collaboration on whole-genome sequencing (WGS) and whole community sequencing funded by Horizon 2020 (https://www.compare-europe.eu/about. The study is currently continued and enriched by epidemiological and ecological modelling and is led by a new centre – Global Surveillance of AMR - funded by the Novo Nordic Foundation Challenge Programme 2017-2023 https://www.globalsurveillance.eu/Projects/Global-Surveillance-of-Antimicrobial-Resistance. As of 2020, 570 sites had been sampled within 102 countries around the world, including Norway, using the same protocol for sewage collection https://www.globalsurveillance.eu/projects/global-surveillance-of-antimicrobial-resistance/protocols-and-procedures.

Hendriksen and colleagues (Hendriksen et al., 2019) from The Danish Technical University coordinated a multi-national study on AMR in untreated wastewater. Metagenomic analysis of untreated sewage was used to characterize the bacterial resistome from 79 sites in 60 countries. The AMR gene abundance strongly correlated with socio-economic, health, and environmental factors and varied systematically by region. They proposed that improved sanitation and health could limit the burden of AMR and that metagenomic analysis of sewage could be a feasible approach for continuous global surveillance of AMR.

The Norwegian project **CORNELIA: Antimicrobial Resistance in One Health Interfaces** focuses on antimicrobial resistance, reducing harmful effects on the environment and uses new technologies for innovation and for the sustainable use and recycling of resources (circular economy). CORNELIA's research approach is based on the idea that wastewater, sludge, and manure constitute a melting pot for interactions between bacteria originating from humans, animals, and the environment. These interfaces are well suited for

the selection and spread of resistance determinants, which may be transferred to soil and water and potentially recycled into food chains. The consortium will develop a scientific basis and strategies for a potential national monitoring programme for AMR in the environment as a parallel system to the existing surveillance programmes in humans, animals, and food. CORNELIA is also working with two start-up companies to test innovative solutions to reduce the amount of AMR bacteria/ARGs that is released into the environment from treatment plants. It is funded by the Norwegian Research Council for the period 2021–2024. https://prosjektbanken.forskningsradet.no/en/project/FORISS/320349?Kilde=FORISS&distribution=Ar&chart=bar&calcType=funding&Sprak=no&sortBy=date&sortOrder=desc&resultCount=30&offset=30&Organisasjon.3=Energi+Norge

NORSE (Network for One Health Resistome Surveillance) aims to create a multidisciplinary network of scientists leveraging their efforts to develop a national One Health resistome surveillance system to monitor the development and dissemination of AMR in animals, humans, the environment, and the food chain. The core objectives of NORSE are to 1) map the needs and requirements for a resistome surveillance system, 2) promote research and develop knowledge on the resistome, transmission of AMR, resistome data collection tools, and clinical applications of resistome data, and 3) promote dissemination of knowledge on AMR in a One Health perspective nationally and globally. (https://app.cristin.no/projects/show.jsf?id=2521986).

Researcher initiatives

Many scientific institutions and scientists have shed light on our limited understanding of the role of the environment in the development and spread of AMR. Hence, their work has resulted in various calls for closing some of these knowledge gaps through monitoring and surveillance efforts. Some examples are provided below.

Huijbers and colleagues (Huijbers et al., 2019) from the Centre for Antibiotic resistance research (CARe) in Sweden presented a conceptual framework for the environmental surveillance of antibiotics and AMR. The different surveillance data should contribute to understanding human, animal, and environmental health risks. They underline the critical importance of defining AMR-linked environmental surveillance objectives and present five key objectives:

- Transmission of already antibiotic-resistant pathogens between humans, animals, and the environment
- Accelerating the evolution of AMR in pathogens through pollution with selective agents and bacteria of human or animal origin
- Surveillance of the impact of antibiotics on ecosystem health
- Surveillance of antibiotic resistance prevalence in human or animal populations through environmental samples
- Surveillance of antibiotic use in human and animal populations through environmental samples

In later work at the same Centre, **Larsson and Flach** (Larsson and Flach, 2021) comprehensively examined current evidence, described risk scenarios, and discussed methods for surveillance as well as drivers and mitigators of risk. The knowledge gaps

examined included the limited understanding of where new forms of resistance emerge with potentially vast consequences for the treatment of clinically important bacteria.

Anjum and colleagues (Anjum et al., 2021) of the JPIAMR network expressed the need to initiate environmental AMR monitoring programmes nationally and globally that complement existing systems in different sectors. They highlight the potential of using *E. coli* as an indicator for the surveillance of AMR in the environment because *E. coli* is also monitored in many current surveillance programmes.

Manaia (Manaia, 2017) explored the risk of transmission of AMR from the environment to humans. The lack of proportionality between AMR abundance and risk was emphasized, and a call was made for a quantitative model to understand the impact of the contaminated resistome. The author outlined key risk-determinant variables and research needs, and the importance of the precautionary principle was highlighted in the absence of concrete risk values. Moreover, the paper showed that methodological limitations impede the opportunity to conduct reliable risk assessments. A follow-up paper addressed in detail the risk of transmission of AMR from endophytic bacteria to humans (Scaccia et al., 2021).

Niegowska and colleagues (Niegowska et al., 2021) from the EC Joint Research Centre in Italy reviewed knowledge gaps in the assessment of AMR in surface waters. They emphasized the need for technical harmonization and optimization of detection methods for environmental concentrations of antibiotics. Harmonized protocols and official guidelines are needed for standardized surveillance for both the toxicity of the compounds (in mixtures) and the acquisition of resistance by susceptible microorganisms in the environment. They emphasized that environmental assessment and monitoring of antibiotics should avoid evaluation of single substances but should consider mixtures.

Paulshus (2020) mapped the occurrence of AMR in wastewater under Norwegian conditions to identify whether local treatment of hospital wastewater could contribute to limiting the dissemination of antibiotic resistance to the environment. Higher levels of antibiotics and ARB were found in wastewater from the hospital than from the general population. Broad-spectrum antibiotics were also present at much higher concentrations in the hospital effluents. The author suggested that "a cost-benefit analysis should be performed to determine whether localized hospital wastewater treatment may be more effective than large-scale implementation at the wastewater treatment plants" (Paulshus, 2020)

Ploy and colleagues, in the editorial overview "AMR in the environment — too complex for surveillance?", highlight the need for additional expertise in addressing environmental resistance (compared to clinical and veterinary settings), including ecologists, evolutionary biologists, environmental scientists, and engineers. Further, it is made clear that the analysis of AMR in the environment is a complex field, placing challenges for the establishment of standardized surveillance methods. Such methods are used in combination including both cultivation of bacteria as well as molecular analyses of environmental bacterial DNA. A remaining prime challenge is our understanding of the environmental factors that govern the spread of AMR in environments affected by climate change, urbanization, and emerging new pollutants (Ploy and Berendonk, 2022).

Pruden and colleagues (Pruden et al., 2021) in the same issue suggested that "now is the time for integrated global surveillance of antimicrobial resistance in wastewater environments". They highlighted the opportunity to build on the momentum-building infrastructure for monitoring of the SARS-CoV-2 virus in sewage and that such efforts also facilitate AMR monitoring. Such an approach can draw on rapidly developing methods, including metagenomics. Methods need to be standardized for sample archiving and data sharing, and they should be modular and made accessible to low- and middle-income countries.

Aenishaenslin and colleagues (Aenishaenslin et al., 2021) explored the global effectiveness of a One Health surveillance programme for AMR. They highlighted three issues that must be addressed in order to identify the added value of One Health-oriented surveillance efforts. These are i) "to better understand mechanisms through which a One Health integrated approach enhances the effectiveness and value of surveillance systems", ii) "more emphasis should be placed on transdisciplinary teams and networks brought together by the One Health approach", and iii) "research is still needed to develop and evaluate conceptual and analytical tools for measuring the degree of integration and estimating the effect of this integration on health outcomes".

6. AMR surveillance in clinical and veterinary settings in Norway and the EU

Different countries have established national surveillance systems to monitor AMR in clinical and veterinary settings. Some systems also include select samples from the food chain. It is noted that these systems have been developed and methodologically refined over decades. They focus mainly on quantifying resistance in known bacterial species of human importance. Consequently, observed changes in resistance levels and types can lead to changes in antimicrobial prescription guidance. Thus, the rationale behind national surveillance is linked to optimized antimicrobial usage and treatment regimes. Here we briefly describe the AMR surveillance systems in place in Norway and the EU.

The Norwegian Ministry of Health and Social Affairs issued a national action plan against AMR in March 2000 that recognized the need for ongoing surveillance and established the NORM and NORM-VET programmes to provide and present data on the occurrence and distribution of AMR over time. From the beginning this was a collaboration between many different governmental sectors (Ministry of Human Health, Ministry of Agriculture and Food, Ministry of Fisheries, and Ministry of the Environment).

Since 2000, an additional four plans/strategies have been issued, the last one in June 2015 (National Strategy against Antibiotic Resistance 2015-2020). The latest strategy launched in June 2015 was intended to expire in 2020, but due to the COVID-19 pandemic it has been extended. The strategy included the explicit targets of reducing antibiotic consumption in humans by 30% by 2020 compared to 2012 and of reducing antibiotic consumption by food-producing terrestrial animals and companion animals by 10% and 30%, respectively, by 2020 with 2013 as the reference year. Additional specific targets in the food production chain are that livestock-associated MRSA does not become established in the Norwegian pig population and that ESBL in poultry production is reduced to a minimum.

From the beginning, surveillance of both human and animal health sectors as well as food production was included, and these plans and strategies recognized the need for ongoing surveillance as a fundamental component of a strategy to combat AMR.

National Strategy against Antibiotic Resistance 2015-2020 (Norwegian Ministries). https://www.regjeringen.no/contentassets/5eaf66ac392143b3b2054aed90b85210/antibiotic-resistance-engelsk-lavopploslig-versjon-for-nett-10-09-15.pdf

6.1 NORM system

The NORM surveillance programme for antimicrobial resistance in human pathogens was established in 1999 and is coordinated by the Department of Microbiology and Infection Control at the University Hospital of North Norway in Tromsø.

NORM is organized in accordance with the NORM Register Regulations and in such a way that safeguards the interests of both the participant laboratories and the users of the NORM register. To achieve this, a professional council (Fagråd) has been established for NORM. The council is tasked with ensuring good professional activity in NORM through its overall medical microbiological and infectious medicine expertise.

NORM is based on a combination of 1) periodic, protocol-defined sampling and testing in primary diagnostic laboratories, 2) annual results from national reference laboratories for specific microorganisms, and 3) the harvest of routine diagnostic results from laboratory information systems. All diagnostic microbiology laboratories in Norway participate in the surveillance system in addition to eleven reference laboratories. For the periodic surveys, isolates are included from defined clinical conditions such as respiratory tract infections, wound infections, urinary tract infections, and septicaemia. All diagnostic laboratories follow the same sampling strategy and use identical criteria for the inclusion of microbial strains. Only one isolate per patient and infectious episode is included unless otherwise stated. All microbes are identified using conventional methods. The surveillance period starts in the beginning of January, and consecutive isolates of specific organisms from specific materials are included for defined time periods. All isolates included in the surveillance protocols are frozen and available for later projects.

Susceptibility testing and quality control

Rapidly growing, non-fastidious bacterial species (*E. coli*, *Klebsiella* spp., *Enterococcus* spp., *S. aureus*, etc.) are examined according to the EUCAST disk diffusion method. Suitable antibiotics are selected for each bacterial species, and the results are interpreted according to the most recent breakpoints from NordicAST, which are harmonised with EUCAST. Specific resistance mechanisms are verified by suitable assays (beta-lactamase production, glycopeptide resistance, ESBL production, MRSA, MLS). Organisms covered by national reference laboratories (various bacteria, including mycobacteria, and yeasts) are examined by relevant methods, either growth-based or genotypic, at the national level. All participating laboratories include appropriate quality control strains and submit the results together with the results for clinical isolates.

Data processing

The specially designed web-based eNORM computer software is used for registration and storage of patient data, sample data, and resistance data. The results are further analysed by WHONET 5.6 with the aid of the BacLink software. In addition, the distribution of microbial species in blood culture is surveyed based on the extraction of routine data from the laboratory information systems of the participants.

6.2 NORM-VET

The NORM-VET monitoring programme for antimicrobial resistance in animals, food, and feed was established in 2000 and is coordinated by the Norwegian Veterinary Institute commissioned by the Norwegian Food Safety Authority. The NORM/NORM-VET reports also present data on the usage of antimicrobial agents in humans and animals in Norway commissioned by the Norwegian Food Safety Authority (Mattilsynet).

Note that the data presented in the NORM and NORM-VET programmes are not directly comparable. This is because the sampling and the classification of resistance differ between the programmes. Clinical breakpoints are used for the classification within NORM, while epidemiological cut-off values (ECOFF) are used for the classification of resistance within NORM-VET.

NORM-VET focuses on AMR detected in clinical isolates from animals and in faecal indicator bacteria from animals and food and in zoonotic and non-zoonotic enteropathogenic bacteria. The selection of bacterial species and respective numbers to be tested vary to a certain extent from year to year and is determined and adapted to relevant national and international issues regarding AMR development and dissemination.

NORM-VET follows the requirements set in the Commission-implementing decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU). In addition, antimicrobial testing of bacteria from other sources than those included in this decision, and the investigation of the presence of specific ARB/resistance mechanisms by selective methods are also included.

Denominator data (animal biomass)

A population correction unit (PCU, where 1 PCU = 1 kg of animal biomass) is used as a denominator and surrogate for the animal population at risk, with categories and calculation methodology identical to, and detailed in, the ESVAC 2016 report. Data on animal populations, including farmed fish, used to calculate the PCU are obtained from Statistics Norway (https://www.ssb.no). Because cattle, pigs, sheep, and poultry accounted for approximately 99% of the Norwegian meat production in 2019 (https://www.ssb.no/slakt) these species as well as goats were selected to evaluate the goals set down in the national strategy. For further details about data quality and estimation procedures, see the NORM-VET reports.

Methods – indicator species

Susceptibility testing of *E. coli* and *Enterococcus* spp. is used as an indicator for the occurrence of AMR in the bacterial population. Selective methods are used for detection of *E. coli* resistant to extended-spectrum cephalosporins, quinolone-resistant *E. coli* (QREC), carbapenemase-producing *Enterobacteriaceae*, colistin resistant (COL-R) *E. coli*, vancomycin resistant *Enterococcus* spp. (VRE), methicillin resistant *Staphylococcus aureus* (MRSA), and *S. pseudintermedius* (MRSP). The use of selective methods is especially relevant for low-prevalence sources because this enables early detection of specific emerging resistance mechanisms such as extended-spectrum cephalosporin-resistant *E. coli* and carbapenemase-

producing *Enterobacteriacae*. Significant reservoirs of such resistant bacteria in animals and the food production chain are of concern because they may interact with human bacterial populations and thus have an impact on resistance development in these populations. Some of these antimicrobials are defined by the WHO as **critically important** for the treatment of human infections. Each antimicrobial agent (or class) is assigned to one of three categories of importance based on two criteria:

a) the agent or class is the sole therapy or one of few alternatives to treat serious human disease, and b) the antimicrobial agent or class is used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources. **Critically important antimicrobials** are those that meet both criteria.

See also EU Regulation 2019/6, 2021/1760 and 2022/1255 for veterinary medicinal products and the criteria behind reservation of antimicrobials for treatment of certain infections in humans.

Methods – susceptibility testing

The substances included in the antimicrobial test panels might not always be those used in veterinary medicine but are included because of their importance for human health. Some of the cut-off values defining resistance applied in NORM-VET have changed over the years, but data on prevalence of resistance presented in earlier reports have been recalculated using the cut-off values applied in 2019. For laboratory procedures analysing samples, see the relevant reports.

EUCAST definitions of clinical breakpoints and ECOFF values can be found at http://www.eucast.org.

For monitoring the usage of antimicrobial agents in animals, including both land-based food-producing animals and farmed fish as well as pet animals, both sales data and veterinary prescription data are synthesized.

6.3 AMR surveillance across human clinical and veterinary settings in the EU and EEA countries

Data collected in Norway on antimicrobial use and the results of antimicrobial susceptibility testing of certain pathogens and indicator bacteria are shared on an annual basis with the EU and analysed and presented in different settings (Table 2).

Table 2. Shared use of Norwegian AMR data in the EU.

Activity	Owner	Focus	Targets	Database	Primary report*
EARS-net	ECDC	Antimicrobial Susceptibility Testing (AST) on blood-culture isolates	*E. coli* *K. pneumoniae* *P. aeruginosa* *Acinetobacter* sp. *S. pneumoniae* *S. aureus* *E. faecalis* *E. faecium*	ECDC Surveillance Atlas AMR	EARS-net AMR Surveillance (annual/biannual)
FWD-net	ECDC	AST in foodborne pathogens (human faeces)	*Salmonella* *Shigella* *Yersinia* *Campylobacter*	Not applicable	The EU Summary Report on AMR in zoonotic and indicator bacteria from humans, animals, and food (biannual)
EFSA reporting	EFSA	AST in isolates from food-production animals	Non-typhoidal *Salmonella, Campylobacter, E. coli* (food-producing animals only), ESBL-AmpC-carbapenemase-producing *Salmonella* and *E. coli* (food-producing animals only), MRSA (meat and food-producing animals only)	Not applicable	
ESAC-net	EMA	Human consumption of antimicrobial substances	Antimicrobials for systemic use (ATC group J01)	ESAC-net database (hosted by ECDC)	ESAC-net Annual report on Antimicrobial Consumption (annual)
ESVAC-net	EMA	Sales of veterinary antimicrobials	Antimicrobials (ATCvet hierarchical system)	ESVAC database	ESVAC annual report on sales of veterinary antimicrobial medicinal products

*Supplementary to these reports, EMA works closely with the EFSA and the ECDC to understand the relationship between the consumption of antimicrobials and the occurrence of AMR. These European Union agencies deliver results of their analyses in the Joint Inter-agency Antimicrobial Consumption and Resistance Analysis (JIACRA) reports.

7. Types and definitions of resistance

7.1 Types of resistance observed

AMR is commonly observed in cultivable bacteria extracted from environmental samples. The occurrence of AMR in isolates from complex bacterial communities can be caused by several mechanisms, including those at the cellular level mentioned below.

Intrinsic resistance: Antimicrobials have selective toxicity so a proportion of bacterial species in heterogenous bacterial communities will naturally not be susceptible to the antimicrobial tested. The concept of intrinsic resistance is important in an environmental context because it defines those microbial species and strains that are naturally resistant and have never been susceptible to the antimicrobial compound in question. Natural resistance can be due to, for example, lack of the target site of the antimicrobial, impermeable cell surfaces, or chromosomally located genes serving other functions but that also confer resistance to particular antimicrobials (e.g. ampC). Because intrinsic resistance is inherently part of the overall physiology of the cell, it is generally not considered to contribute to observations of newly acquired resistance in microbial populations and communities.

Hetero resistance: Most antimicrobials only affect metabolically active microbial cells. Cells in a dormant state or spores will thus not necessarily be affected. Larger bacterial populations may contain cells in various physiological states, and the concentration and duration of antimicrobial exposure will thus determine to what extent inactive members of a bacterial population may survive a temporary exposure to antimicrobials. Some antimicrobials only exert bacteriostatic effects, preventing growth rather than killing the microbe as a bactericidal. The outcome of a time-limited antimicrobial exposure may thus in some cases not be fully lethal to the overall population.

Acquired resistance: Of concern to this opinion is the dynamics of acquired resistance in the environment. Resistance in previously sensitive bacterial populations can occur through two means – i) mutations where random genetic processes within the bacterial cells lead to phenotypic changes that reduce or abolish the bacterium's sensitivity to the antimicrobial, or ii) through horizontal acquisition of genetic material from other cells that upon expression will reduce or abolish the bacterium's sensitivity to the antimicrobial.

Co-resistance: Resistance occurring when the genes specifying different resistant phenotypes are genetically linked or present in the same cell, for example, by being located together on one or more mobile genetic elements.

Cross-resistance: Resistance occurring when the same or similar mechanism(s) of resistance applies to different antimicrobials, for instance multidrug efflux systems.

Induced resistance: Intrinsic or acquired resistance that is not expressed in a continual manner but is triggered/induced by specific environmental factors.

The resistome usually refers to the overall presence of genetic resistance determinants in a bacterial community sample identified after DNA sequencing. The extent to which the

different phenotypic resistance types described above is part of a resistome map will vary with the community being sampled.

7.2 Clinical definition of resistance

Most phenotypic methods for antibiotic susceptibility testing of bacteria (and some other microbes) are based on inhibition of (visible) growth of the bacteria in a standardized test system in the laboratory. In Norway the methodology described by NordicAST/EUCAST (European Committee on Antimicrobial Susceptibility Testing) is used.

Some important definitions in antimicrobial susceptibility testing are the following.

MIC: Minimum inhibitory concentration (the lowest concentration of an antibiotic able to inhibit (visible) growth of a bacterium). The test medium, inoculum density, temperature, antibiotic concentrations, etc., are standardized (ISO 20776-1 document). How the result is reported depends on the microbe and the context for testing.

In human medicine the result of a test is mostly reported as a categorization using the letters S, I, and R relating to the anticipated rate of success when using a specific antibiotic against a specific bacterium in a clinical infectious disease setting. EUCAST: New S, I and R definitions (https:// www.eucast.org/newsiandr/) Accessed 2021 12 06.

- **S** - Susceptible, standard dosing regimen: A microorganism is categorised as "Susceptible, standard dosing regimen" when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
- **I** - Susceptible, increased exposure: A microorganism is categorized as "Susceptible, Increased exposure" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or its concentration at the site of infection. Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, and distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.
- **R** - Resistant: A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

Clinical breakpoints

The clinical breakpoint is the MIC value of an antibiotic that is effective for a given pathogen in more than 90% to 95% of cases in patients with infectious diseases. Breakpoints assist in determining if an antibacterial is potentially useful in the treatment of a specific bacterial infection, and they are set by EUCAST in the EU.

MBC: minimum bactericidal concentration

The MBC is the minimum concentration of an antimicrobial drug that is bactericidal. It is determined by re-culturing (subculturing) broth dilutions that inhibit growth of a bacterial organism (i.e., those at or above the MIC).

ECOFF: epidemiological cut-off value is often used when there is no or too little evidence behind a test result and clinical response rate. The ECOFF is useful for detecting microbes with acquired resistance from those without such resistance (also called wild type microorganisms). The EUCAST definition of an ECOFF is the ability to distinguish

microorganisms without (i.e. wild type) and with phenotypically detectable acquired resistance mechanisms (i.e. non-wild type) to the agent in question.

ECOFF is typically often used in veterinary medicine and for some environmental bacterial isolates to distinguish wild type organisms from those with acquired resistance mechanisms. In veterinary medicine this may signal that the microbe (probably) is susceptible to a suitable for the infection and animal in question.

Minimum selective concentration (MSC) is a useful concept for understanding the selective effects of antimicrobials in the environment. MSC is related to MIC. The MSC is, however, used to describe the minimum concentration of a compound that exerts a selective effect at the sub-lethal level. MSC values have been reported to be in the range of 1/230 to 1/4 of the MIC (Gullberg et al., 2011).

7.3 Environmental definition of resistance

The clinically used resistance taxonomy and methods as outlined above have been developed over decades for the human and animal/veterinary pillars of One Health. Few of these historical and operational conditions apply when attempting to describe fluctuating resistance patterns in environmental communities (Singer et al., 2016). The main reason is that clinical resistance descriptors are developed in the context of antimicrobial drug therapy, i.e., resistance is calibrated to describe situations where the pathogenic microbe grows at higher concentrations of the antimicrobial than what can be achieved in a human/animal therapy setting. In contrast, most environmental isolates do not cause disease, and descriptors of changes in their resistance levels cannot be categorized within a clinical outcome/organismal therapy context.

Defining resistance in single environmental isolates

The high number of species present in environmental samples will require some prioritization of the species considered. In cases where the environmental bacterium in question can be grown in pure culture in the laboratory (as an isolate), the methodology developed for describing the level of sensitivity to a given antimicrobial for clinical isolates can be followed, and this includes the use of MIC and ECOFF values.

This approach is useful, for instance, for resistance determination in culturable bacterial species with a recognized pathogenic potential that resides in the environment. These species include zoonotic bacteria, many waterborne bacteria (including those present in sewage and manure), and other bacteria for which humans may be accidental hosts:

a. bacteria recovered from the environment that were originally dispersed from human/animals/farms (e.g. through sewage and manure)
b. bacterial strains similar to human strains found in vertebrate gut microbiomes (e.g., *E. coli*, *Enterococcus, Klebsiella*)
c. bacteria with shared ecology, including those causing zoonoses (e.g., *Salmonella, Campylobacter*, see NORM-reports)
d. bacteria that are non-human pathogens (*Some Streptococcus species*- pets/dogs, see NORM-VET)

e. known environmental pathogens (e.g., *Vibrio, Shewanella, Aeromonas, Pseudomonas*) and opportunists (e.g., *Acinetobacter* spp., *Pseudomonads*, *Stenotrophomonas*).

Common to these species is that they can be isolated, cultured, and approached from a technical point through the established clinical resistance methodological framework. Many, such as environmental pathogens, have their main lifecycle in other environments than human and animal hosts (https://www.ncbi.nlm.nih.gov/books/NBK560445/). As considered below, approaching bacterial species and cells of the unculturable fraction of bacteria is currently severely constrained from a methodological perspective.

Defining resistance in bacteria present in complex environmental samples

Samples from spatially heterogeneous and fluctuating environments such as soil, sediments, and the gastrointestinal tracts of (in)vertebrates contain microbes that cannot easily be obtained in pure culture. Moreover, they are likely to contain microbial species that are yet to be characterized at the genera/species/strain level, with unknown resistance history, and with a sizeable fraction that cannot be cultured. For instance, less than 1–10% of soil microbes are considered culturable.

For the culturable bacterial fraction, selectable media may be available for only some species. Hence, only a minor fraction of the bacteria present can be subject to the susceptibility methods available for clinical/single environmental isolates as outlined above.

Methods to describe resistance changes in the non-culturable fraction of bacteria in complex environmental samples have yet to be defined and agreed upon at the international level.

Several proxy measurements could be considered for non-culturable bacteria and or as generic descriptors of complex samples for which characterization of single and culturable isolates would not be considered representative of the sample.

These proxy measurements can be:

Culturable resistant fraction/genomes versus total culturable fraction/genomes
Genotypic characterization of cells or metagenomic samples

- Single-cell genomics
- Metagenomic approaches to extracted DNA
    - Prevalence of specific resistance genes
    - Prevalence of genetic resistance gene patterns
    - Resistance gene patterns linked to host genomes
    - Resistance patterns linked to mobile elements
    - Presence of mobile genetic element "vectors"
- Metagenomic approaches to extracted RNA
    - Level of RNA expression of specific resistance genes
    - Level of RNA expression of patterns of resistance genes
- Cell/isolate or meta sample approaches to protein content

The methods used to study phenotypic resistance traits and the genetics and diversity of resistance traits present in an environmental sample are outlined in chapter 8.

7.4 The panel´s consideration of a definition of environmental resistance

The overview provided above, as well as further detailed examination in chapter 8, suggests the following.

Definition of resistance in single environmental isolates

For environmentally derived bacterial species and strains that can be cultured as isolates in the clinical laboratory, the currently available phenotypic measures such as MIC, MBC, and MSC will apply.

For bacterial species/strains with known history of pathogenicity, the level of antimicrobial susceptibility and clinical breakpoints (e.g., S-R) can be described following routines established in the clinical settings of NordicAST/EUCast and ECOFF. The ECOFF may be of particular value for those isolates with limited understanding of pathogenicity and clinical relevance.

Resistance descriptors for complex environmental samples

The approach to describing resistance characteristics in complex environmental samples will differ from those focused on single isolates. The shift from single isolates to bacterial communities also suggests a shift from an unambiguous, isolate-focused *Definition of resistance* to identifying a uniformly recognized *Description of resistance*. For the latter, a well-defined set of descriptors may facilitate longitudinal monitoring efforts with data that can be shared across sampling sites and contexts. Such descriptors may be useful to shift our current point prevalence-focused approaches to a more holistic and longitudinal approach focusing on changing patterns of resistance over space and time due to drug usage patterns, dispersal and dissemination mechanisms, and interventions.

In conclusion, and with reference to Mandate question 1.

For single microbial isolates, the definition, taxonomy, and clinically-oriented methods developed for identifying and describing the level of antimicrobial susceptibility (MIC, MBC, MSC) can be directly applied to culturable environmental isolates. For those environmental isolates with a known history of causing disease (e.g. zoonoses, waterborne pathogens), resistance acquisition/development and dynamics can be expressed through clinical breakpoints and ECOFF values.

For observing and communicating changing patterns of resistance in complex microbial communities and environmental samples, a new set of descriptors must be developed, standardized, and harmonized at the global level. Such descriptors should be developed based on a clear taxonomy and standardized methods and reporting formats. The descriptors seek to describe the resistome present in the overall microbial community rather than resistance traits present in single cells.

Building knowledge on environmental resistance in microbial communities may therefore not rely a new or redefined definitions, but rather on how the descriptors are defined and their implementation in surveillance efforts.

8. AMR in the environment – methods

Numerous methods are available to describe AMR in microbial communities present in environmental samples such as soil, sediment, water, air, etc. The choice of sampling sites and types will depend on the scope of the investigation.

Several considerations must be made prior to collecting resistance data from environmental settings:

- Identifying and describing the unresolved knowledge gaps (e.g., prevalence, incidence, associations, causality, effect of interventions, exposures, etc.)
- Formulating the specific knowledge gap and problem addressed and the corresponding environmental site(s)
- Designing the overall study (e.g., point prevalence versus longitudinal orientation)
- Describing the known baseline, biology, and (exposure) history of the sites studied
- Identifying and describing the opportunities for comparators and time series
- Determining the specific number of sampling sites and their sensitivity and depth
- Selecting standardized methods and data reporting formats

A key consideration is the type of environmental sample/bacterial fraction considered for further resistance characterization. The characterization of resistance can be divided into three different groups:

I) Characterization of resistance in single isolates of culturable bacteria with known history/established biology and relevance.

- bacteria recovered from the environment that were originally dispersed from humans/animals/farms (e.g. through sewage and manure)
- bacterial strains similar to human strains found in vertebrate gut microbiomes (e.g., *E. coli*, *Enterococcus, Klebsiella*)
- bacteria with shared ecology, including those causing zoonoses (e.g. *Salmonella, Campylobacter*, see NORM),
- bacteria that are non-human pathogens (*Streptococcus* - pets/dogs, see NORM-VET) and those infecting wildlife
- bacteria that are known environmental pathogens (e.g. *Vibrio, Shewanella, Aeromonas, Pseudomonas*) and where humans/domestic animals are accidental hosts (e.g. *Acinetobacter*, *Pseudomonads*, *Stenotrophomonas*, etc.).

These are today the most common targets for resistance characterization. Because they can be obtained as single isolates, the methods used to determine AMR are the same as those developed for clinical/pathogenic isolates from humans and animals.

II) Characterization of resistance in single isolates of culturable bacteria with unknown history/un-established biology

- Most isolates from soil/water

Resistance characterization in such samples can also draw on methods developed in a clinical context. However, the interpretation of the resistance patterns observed will often lack the

comparative framework needed to understand changes in susceptibility over time. Moreover, the direct clinical relevance may not be obvious in many cases. In many cases, the ECOFF values can be used to describe susceptibility.

For these first two sample and isolate choices (I and II), resistance determination usually starts with phenotypic methods including growth assays on selective media followed by the opportunity to identify proteins (MALDI-TOF) and/or extract DNA for further analysis of traits (PCR) or whole genomes (genotyping methods).

III) Characterization of resistance in complex environmental samples that include the isolates described above (I, II) as well as the non-culturable fraction of microbes

- DNA extracted from composite and or pooled samples

For this latter sample type, characterization of AMR is indirect and is based on the presence of known resistance genes in the extracted DNA. Results are most often presented as tables of identified resistance genes with relative (metagenomics) or absolute (qPCR) occurrence in samples of relevance. Information on the functional phenotype (e.g. MIC levels) produced by the identified resistance gene/trait are rarely available. Other omics-based approaches such as transcriptomics and proteomics may help reveal resistance gene expression patterns.

8.1 Phenotypic methods (culture-based methods)

The characterization of AMR in culturable bacteria usually starts with the isolation of the single bacterial cells/cultures from the sample material. This is usually done by inoculating a portion of a suspended sample on a growth medium, usually solid, that favours the growth of the bacterial species of interest, under suitable environmental conditions (temperature, oxygen availability etc.). The traditional, culture-based approach for assaying AMR is known as antimicrobial susceptibility testing (AST). In this approach, a microbial isolate is grown in the presence of an antimicrobial substance at different concentrations. The lowest concentration that inhibits growth is known as the MIC. This approach is well established in clinical settings, and the MIC values can be related to clinical breakpoints and therapy settings (Mouton et al., 2012).

AST methods can also be used to determine MIC values for bacterial isolates from environmental samples. Depending on the isolate in question, the observed MIC value may or may not be interpreted in the context of clinical breakpoints.

Obtaining isolates in pure culture is often the basis for further detailed genetic characterization of observed resistance phenotypes. However, only a small proportion of the bacterial diversity found in the environment can be cultured in the laboratory (Lloyd et al., 2018). The resistance characteristics of such species/strains can today only be understood through indirect genotyping methods.

8.2 Genotyping methods (culture-independent methods)

Genetic characterization focuses on the trait/anticipated gene of interest by gene sequencing (genetics) or whole genome sequencing (genomics), PCR techniques, or shotgun metagenomic sequencing of environmental samples. The genetic/genomic approach relies on

single isolates, whereas the metagenomic approach is focused on composite samples and entire communities. It is noted that AMR genes only constitute a minor proportion <0,5%) of any total DNA extract. Sequencing depth is therefore essential when non-targeted methods are used. PCR approaches are amenable to both cases.

Single gene/isolate focus

Gene-based techniques such as PCR assays are usually quick and rely on the set number of candidate/target genes chosen. The method can identify known resistance markers as well as determine the genera/species of the isolate in question. Subsequent DNA sequencing of the amplified PCR products allows higher resolution of the resistance genes identified and multi-locus sequence type patterns for recent phylogenies of the bacterial isolate.

Multiplexed PCR reaction protocols have also been developed to allow for simultaneous detection of several resistance genes (Sigmund et al., 2020; Strommenger et al., 2003)

Single gene/metagenome focus

The DNA sample targeted for directed molecular approaches (e.g., PCR) can be extracted from single cells in culture or from whole microbial communities. In the latter case, the overall prevalence of bacterial genera/species (16S rRNA gene-based) and resistance determinants (specific target genes) can be determined. It is, however, somewhat challenging to link the observed resistance genes to specific members of the bacterial community examined.

The PCR-based approach is considered relatively sensitive, and quantitative PCR (qPCR) protocols have been developed for many resistance markers (Røken et al., 2022; Zhu et al., 2017). On the other hand, the sensitivity of qPCR will depend on the target, the quality of the DNA extract and the specific methodological set-up used.

The number of bacterial genomes targeted in a single PCR is usually below 10,000 genomes/bacterial cells. This is a limitation to the assays if a few bacterial species numerically dominate the sample (Nielsen and Townsend, 2004).

Whole genome/isolate focus

AST is often followed by whole genome characterization of resistant isolates to pinpoint the mechanism of resistance (Argimón et al., 2020). Within a species of bacteria, AMR profiles can vary extensively (Anderson et al., 2006) and WGS can help identify and characterize novel or dominant strains of particular concern. WGS is also a valuable tool for tracking transmission of resistance genes and their mobile units among lineages and populations of microbes (Salamzade et al., 2021).

Whole genome/metagenome focus

The development of next-generation sequencing techniques has facilitated a shotgun metagenomics approach that seeks to describe the environmental resistome (the totality of AMR genes in an environment) (Nayfach et al., 2021). This approach is based on first

extracting the total DNA from an environmental sample and sequencing that DNA as if it represented a single genome, but at very high sequencing coverage. Recent advances in genome assembly techniques allow for massive amounts of short read (e.g. Illumina) data to be supplemented with data from long-read technologies (PacBio or Nanopore) (Bertrand et al., 2019). Computational tools are used for assembling the sequence reads into contigs, i.e., longer continuous stretches of DNA sequence, and to further separate contigs into putative genome sequences often referred to as metagenome assembled genomes (MAGs). The MAGs can then be searched against a database of known resistance gene sequences. There are also methods that circumvent the computationally intensive process of MAG assembly by mapping raw sequence reads directly to an AMR database. This approach can be effective for detecting genes carried on mobile elements, like plasmids. However, this approach will not provide information on the taxonomy of the organisms harbouring the genes (Sivalingam et al., 2020). The criteria for gene identification may vary between studies and create variability and affect comparability

There are several databases available of DNA sequences linked to resistance traits. These databases may differ in the criteria they use for inclusion, e.g. intrinsic versus acquired resistance traits, accessibility, level of customization available etc., that may lead to variation between studies.

- The comprehensive antibiotic resistance database (CARD) is a curated collection of resistance gene sequences, including single nucleotide polymorphism gene variants that have been linked with resistance (Alcock et al., 2020). CARD is extensively annotated and implements its own antibiotic resistance ontology system for the characterization of genes, gene products, and resistance mechanisms. It is accompanied by the Resistance Gene Finder software tool, which is generally geared towards searching for ARGs in whole genome sequences and MAGs.
- ResFinder (Bortolaia et al., 2020) has a database that is focused on acquired resistance and is accompanied by custom search tools, including a web-based implementation that can use both genome assemblies and unassembled reads.
- The BacMet database focuses on genes conferring resistance to antimicrobial biocides and metals (Pal et al., 2014).
- MEGARes combines data from several databases, including CARD ResFinder and BacMet in a format that is suitable for searching with unassembled metagenomic reads (Doster et al., 2020).
- There are also databases that are specific to bacterial species (Coll et al., 2015) or classes of antibiotics (Bush and Jacoby, 2010).

Environmental DNA/metagenome focus

Extracts of DNA from complex environments such as soil, sediments, water, and faeces consist of DNA from live cells as well as extracellular DNA shed from decaying cells and organisms. Extracellular DNA in the environment will be present as fragments in different states of decay. Bacteria are also known to shed DNA during growth (e.g. in biofilms) and upon decay. Such DNA will therefore also contain resistance genes present in the host microbe's extracellular DNA (Sivalingam et al., 2020). Extracellular DNA can also retain biological activity e.g. after being taken up by bacterial cells through natural transformation (Kittredge et al., 2021).

The extracellular DNA fraction in the environment has received increasing attention because it may persist over times longer than the lifespan of the host organisms. Moreover, such DNA may become dispersed in the environment, e.g. through river systems, to sites far from the host. The focus on environmental/extracellular DNA has now emerged as an independent research field with its own scientific journals https://onlinelibrary.wiley.com/journal/26374943 and society https://ednasociety.org. The methodological developments in the field of environmental DNA are likely to benefit environmental surveillance of AMR as well.

Metagenomic methods can be applied to such extracts as well. The DNA extracts from environmental DNA may differ (in the sampling and extraction protocols) from DNA extracts targeting the bacterial DNA fraction. Nevertheless, both types of extracts will contain DNA that originate both from extracellular DNA present in the sample and that released from lysed microbes.

Functional metagenomics

Another approach to AMR detection is known as functional metagenomics, which combines phenotype and gene-based methods. This approach leverages the cloning of random fragments of extracted DNA onto an expression vector and transformation into a cultivable antimicrobial-susceptible bacterial strain. Thus, this approach can be thought of as culture independent with regard to the original host of a given DNA fragment. The newly transformed host strain can then be assayed for newly acquired resistance using the standard susceptibility testing. The cloned DNA fragments will be present as vector inserts in the resistant cells and can be sequenced to characterize the genetic elements conferring resistance. This approach has shown potential in the discovery of novel resistance genes (Riesenfeld et al., 2004).

Transcriptomics

Transcriptomics is the study of the complete set of all the ribonucleic acid (RNA) molecules expressed in some given biological entity, such as a cell, tissue, or organism. Classes of RNA molecules include messenger RNAs (mRNAs), transfer RNAs, ribosomal RNAs, microRNAs, and different types of long noncoding RNAs (Milward et al., 2016). The main usage of transcriptomic techniques is to study gene expression patterns by analysing the complement of mRNA molecules in biological samples. Currently, the most common approach to gene expression analysis is reverse transcription of extracted mRNA molecules, followed by high-throughput DNA sequencing. This allows researchers to evaluate how cells and tissues respond to varying environmental or experimental conditions and to assess which parts of their genomes are being actively transcribed. Transcriptomic techniques have also been adapted and applied to environmental samples of microbial communities (Zhang et al., 2021), an approach dubbed metatranscriptomics.

While metatranscriptomic techniques show great promise, there are still some major caveats. First, mRNA molecules have inherently short half-lives and specialized sampling procedures are required to obtain useful sample material. Especially during field sampling this can be a complication. Second, samples tend to be dominated by structural RNA molecules, particularly rRNAs, that are usually of lesser interest and can swamp target mRNAs during sequencing analysis. In the case of AMR gene surveys, an additional drawback is that non-essential genes, like AMR genes, are only expressed under certain conditions (induced

resistance). Thus, only looking at actively transcribed genes entails a risk of not detecting AMR genes that are present, and even prevalent, in an environmental sample.

8.3 Non-DNA based methods

Proteomics

Isolate focus

MALDI-TOF MS has emerged as a quick and reliable approach for bacterial identification, even for microorganisms that are difficult to culture. Test modifications have been developed to improve sensitivity and accuracy to include detection of AMR phenotypes. This is achieved by detection of AMR proteins, modifications, or breakdown products of the target antimicrobial substance or inhibition of bacterial growth in the presence of antimicrobials (Biswas and Rolain, 2013; Choquet et al., 2018; De Carolis et al., 2017; Idelevich et al., 2018)

8.4 Methodological limitations

Various methodological limitations are known both from the phenotypic as well as genotypic approaches to resistance characterization. A major drawback of the phenotypic approach to describing AMR profiles in environmental samples is that in many cases the bacteria is not culturable, or the genera or species are novel and remain to be described. These are currently described as operational taxonomic units or amplicon sequence variants. The limited knowledge of current species diversity in environmental samples limits the extent to which typical indicator species/baselines are available. The lack of established history of many isolates limits the opportunity to understand changes in the resistance pattern over time because such changes depend on the availability of a baseline or a comparator. A major drawback of gene-based methods for AMR gene detection is that AMR discovery is restricted to known marker sequences available in current databases. The contents of AMR databases are biased toward human pathogens and model organisms, neglecting most microbes that are found in the environment. Model-based approaches have been developed to mitigate this bias (Gibson et al., 2015), but these come with their own uncertainties. Inclusion criteria for resistance gene sequences in databases vary, and they will differ/expand over time. Hence, studies conducted at different time points will therefore compare a given data set to the different database/sequences.

Another limitation in metagenomic approaches is the limited opportunity to assign identified resistance genes to the host cell and species. The heterogeneous distribution of resistance genes on mobile genetic elements (e.g. plasmids, transposons) in more than one host species/subpopulation in the sample may further complicate host cell assignment (Buta-Hubeny et al., 2021).

Other constraints

Although not a methodological limitation per se, the history of the sample investigated may not be fully known. For instance, the prior use of manure or the type of irrigation water used in the field where a soil sample is taken is highly likely to influence the resistance pattern observed and the conclusions drawn.

The point prevalence-oriented sampling approach that dominates today also misses the temporal dynamics of the resistance traits considered. The representativeness and "shelf-life" of such data is therefore in most cases not known.

Table 2. Advantages and limitations of different methods for determination of resistance in environmental bacteria

Method	Target cells/population	Advantages	Limitations
Culture-based methods*	Single bacterial isolates that can be grown in pure culture	- Using epidemiological cut-offs based on population MIC distributions or ecological cut-offs based on arithmetic MIC distributions -Can reliably detect resistance phenotypes conferred by novel ARGs. The taxonomy and the genetic basis for the observed resistance phenotype can be further determined by molecular methods (PCR, WGS) -Observed resistance phenotype is directly linked to the genotype -Isolate can be further characterized through molecular methods, including PCR of target genes and whole genome sequencing.	-Most bacteria from the natural environment cannot be cultured in the laboratory, a limitation that is particularly profound in environmental samples. - Many bacteria can enter a state where the microbe is alive but does not multiply under environmental stress (viable but non-culturable) -For bacteria that can be cultured, the process can be time-consuming, requiring long incubations, multiple steps, and confirmatory analyses. -Methods used to store the samples and the duration of storage can both strongly influence the recovery and quantification of the target organisms. -MIC cut-offs to determine susceptibility are based on clinical treatment outcomes and may not be appropriate for environmental monitoring -MIC performs at clinically relevant standard temperatures, which may not reflect environmental conditions -Most environmental samples contain high numbers of bacteria per gram of sample material, and sampling efforts become quickly saturated. -Most environmental samples contain a high number of bacterial species in contrast to many clinical specimens.

Method	Target cells/population	Advantages	Limitations
Culture-independent methods **<u>DNA based</u>** **Molecular based** **(Single gene targets)**	Gene-based (single R gene targets_PCR) genomics-comparative genomics of known strains of the same species	-Faster than culture-based methods and can detect the presence of ARGs even in bacteria that are difficult to culture in the lab. -Allow fine-scale molecular epidemiology of resistance genes/gene transfer vectors taking into account that the evolution of resistance genes may be different from the host cell	-Detection of the gene is not equivalent to resistance as defined by clinical standards because genes are not always expressed -An ARG is detected in a sample, or even in a bacterium, does not mean it translates to expressed resistance or organism viability. Therefore, resistance genes are indicators of the genetic potential for resistance, not explicitly resistant bacteria. -Not all resistance traits are described as sequences in the current R-gene databases
Metagenomics	-Large-scale genomics applications as a way to study the taxonomic and functional composition of microbial communities -Comparative bioinformatics	-Methods have the ability to detect many different resistance and non-resistance genes present in a sample in a single metagenomic-sequencing run (PCR-based methods require a separate test for every specific gene of interest). -Reliably detect resistance-conferring novel ARGs.	-Expensive -Quantification is limited to proportions rather than absolute numbers of resistant organisms. -Sensitivity can be limited and may vary significantly, because reads for specific genes are only a small proportion of the total number of reads. -The method cannot detect novel ARGs that do not resemble previously identified genes and might misclassify genes that have acquired activity against new drugs (e.g., the acquisition of quinolone activity by aminoglycoside acetyl transferases).
<u>Protein-based</u> **MALDI-TOF-MS** (Vrioni et al., 2018)	- Detection of gene products (proteins)	-Detection of AMR proteins -Detection of multiple AMR at the same target -Suitable for automatization and directly from clinical specimens	-The technology needs further development of research protocols that will be validated for routine application.

*Reference: https://wellcome.org/sites/default/files/antimicrobial-resistance-environment-report.pdfference

9. Antimicrobial agents

Antimicrobial agents is a general term used for chemical compounds that either kill or inhibit the growth of microbes. The concept of antimicrobials applies to antibiotics, preservatives, disinfectants and sanitizing agents, and biocidal products in general. Antimicrobial agents also include antifungal agents and PTMs. Some herbicides may also have antimicrobial activity (e.g. glyphosate). Whereas the modes of action of antibacterial and antifungal agents are based on the effect on one target, the mechanisms of action of PTMs and biocides are based on multiple targets or general toxicity in bacteria. For a definition of different antimicrobial agents, see the "glossary" section of this report. In this assessment, we focus on AMR in bacteria as a response to antibacterial agents and not to anti-fungal agents, PTMs, or disinfectants. The role of these latter compounds was discussed in the VKM report "Assessment of the impact of wastewater and sewage sludge treatment methods on antimicrobial resistance" (VKM et al., 2020).

In this chapter we briefly describe the usage patterns in Norway, decay rates, and methods of detection.

9.1 Usage patterns of antimicrobial agents in Norway

Data from NORM/NORM-VET 2020

Animals

The total sales of antibacterial veterinary medicinal products (VMPs) for terrestrial animals in Norway was 5019 kg in 2020. Sales of antibacterial VMPs for use in terrestrial food producing animals, including horses, were 4659 kg in 2020. From 2013 to 2019, the sales of antibacterial VMPs for cattle, pigs, poultry, sheep, and goats declined by 23% when measured in kg and by 18% when measured in mg/PCU (population correction unit).

In 2020, the sales of antibacterial VMPs for farmed fish (cleaner fish included) was 223 kg. This is a reduction of more than 99% compared to 1987, when the sales were at their highest (almost 50 tonnes). During the same time there has been a 20-fold increase in the biomass of farmed fish.

The sales of antibacterial VMPs marketed for companion animals were 360 kg in 2020, a reduction of 32% since 2013. Data from the Veterinary Prescription Register show that prescriptions of human antibacterial medicinal products also declined gradually by 21% (kg) during this period.

Norway has very little use in animals of "restricted antibacterial classes", as suggested by the European Medicines Agency (EMA). The use of Narasin as a coccidiostat feed additive in the poultry industry was stopped in 2016. The usage of therapeutic antibiotics for broilers continues to be very low in Norway, and in 2020 only two broiler flocks were subjected to one such treatment each and only beta-lactamase susceptible penicillins were used.

Sales data

Wholesalers and feed mills in Norway are required to provide sales statistics for antibacterial VMPs. Medicated feed is supplied to the end user by feed mills currently only for farmed fish. Obtaining, quality controlling, analysing, and reporting the data is the responsibility of the Norwegian Veterinary Institute (NVI) in collaboration with the Norwegian Institute of Public Health (NIPH). This includes sorting the data into sales of antibacterial VMPs for terrestrial animals included in the human food chain (including horses) and those approved solely for companion animals, as well as antibacterial VMPs used on special permit (products approved in another European Economic Area (EEA) country – amounting to 2% of Norwegian sales). Antibacterial VMP sales for food-producing animals have been further stratified into those for treatment of individual animals and for group treatment.

Human medicinal products (HMPs) can also be prescribed for animals if there is no VMP authorised for the condition (Directive 2001/82/EC, Article 10). For food-producing species it requires that a maximum residue level has been assigned or found unnecessary for the active substance.

Usage data

The Norwegian Food Safety Authority (Mattilsynet) established the Veterinary Prescription Register (VetReg) for farmed fish in 2011 and for terrestrial animals in 2012. Veterinarians are required to report use administration and deliveries of VMPs and HMPs for all terrestrial food-producing animals and horses to VetReg, while reporting is voluntary for other animal species such as companion animals. Pharmacies and feed mills have to report all deliveries, including medicines prescribed for companion animals and HMPs.

The usage patterns represent the data reported to VetReg by farmers, pharmacies, and veterinarians. These reports are known to be incomplete, but predictably so, and considerable statistical work has been put into estimating the real usage of different compounds in different settings and matching them with the sales data.

The classification system is identical to the inclusion criteria by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC).
https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-31-european-countries-2019-2020-trends-2010-2020-eleventh_en.pdf

EU regulation 2021/578 outlines the requirements for the collection of data on antimicrobial medicinal products in animals.

Humans

The total antibiotic use here means all sales of antibacterial agents for systemic use in humans (ATC class J01, excl. methenamine1) both in primary care and in health care institutions. Use is measured as defined daily doses (DDD)/1,000 inhabitants/day. The total sales were 11.5 DDD/1,000 inhabitants/day in 2020. According to the data from NORM-NORM/VET (2020), there has been a reduction of 32% in total antibiotic use since 2012.

Primary care prescriptions account for approximately 84% of human use of antimicrobials. In primary care, the most important antibiotic group in 2020 was penicillins (J01C), making up 37% of the DDD and 52% of the prescriptions in ATC group J01 excluding methenamine, followed by tetracyclines (J01A) at 19%. The three most prescribed antibiotics for outpatients in 2020 were phenoxymethylpenicillin, pivmecillinam, and doxycycline. These three substances represented 49% of all prescriptions and 52% of all DDD sold. The proportion of narrow-spectrum penicillins (J01CE) was stable from 2012 and accounted for 27% of total sales (J01, excl. methenamine).

Hospital use accounted for 8% of total sales of antibacterials to humans in 2020. Compared to 2019 a decrease of 11% in DDD/1,000 inhabitants/day was observed. Measured as DDD/100 hospital bed days, an increase of 14% has been observed since 20212. During the same period, only a slight increase of 1% in DDD/admissions has been observed. The use of broad-spectrum antibiotics was reduced by 20% compared to 2012 (measured in DDD/100 bed days). Penicillins (J01C), measured in DDD/100 bed days, accounts for about half of the antibiotic use with cephalosporins as the second largest group with 20% of all DDD/100 bed days.

9.2 Dispersal and decay

Man-made antimicrobials are produced in vast amounts on a global scale and disperse from their site of production as well as from the site of intentional use. Such pharmaceutically-produced chemicals are thus considered pollutants when present at sites not intentionally exposed to them. These antimicrobials may exert a positive selection pressure on microbial communities present at distant sites and at different time intervals from the intended usage (Larsson and Flach, 2021). Some antimicrobials are also produced naturally at low concentrations (Box 2, see below).

The most important dispersal pathways of antimicrobial agents include through urine and faeces from humans and animals; run-off from farms, plant orchards, or aquaculture; and via waste streams from the production sites of antibiotics (Larsson and Flach, 2021). Post-treatment excretion of antimicrobials may reach levels that contribute to resistance development in environmental bacteria, including pathogens. This phenomenon, which is

[1] Methenamine is a urinary antiseptic and is not considered an antibiotic. The drug may spontaneously be converted to the antiseptic formaldehyde in the urinary bladder. Methenamine accounted for 25% of all DDD in the antibacterial J01 group.

known as the eco-shadow concept, is considered a new way of following the environmental impacts of antimicrobials (Bengtsson-Palme and Larsson, 2016; Kookana et al., 2014; Midtvedt, 2001).

Both biotic and abiotic factors like temperature, solar radiation exposure, pH, soil type, and microbial biocomplexity may influence how long antimicrobial residues remain in the environment (FAO, 2018; Kallenborn et al., 2008). Many antimicrobial agents decay over time in most environments due to biological, chemical, and physical processes. Exception are semi-synthetic and synthetic anti-microbial agents like quinolones and tetracyclines, which are more stable in the environment due to their chemical properties (Yang et al., 2021).

The environmental persistence of antimicrobials can also rationally be explained by their having been specifically developed to retain activity and to resist degradation by the biochemical activities of the human body and exposed microbes (Kallenborn et al., 2008). Although not the focus of this report, other compounds with antimicrobial effects such as some biocides can have long decay rates, and PTMs are even non-degradable. For more information regarding the stability of antimicrobial agents, see our previous report (VKM et al., 2020)

Box 2 Natural occurrence of antimicrobials/analogues

Identical or near identical versions of some pharmaceutically-produced antimicrobials may be naturally present in a sampled environment. Naturally-occurring antimicrobials are considered part of the parvome (the collective set of all small molecules produced by organisms) of microbe communities, playing roles in antibiosis, signalling, metabolism, and quorum sensing. The microbial production of such compounds at the sites sampled is, however, not considered significant in the quantification of man-made antimicrobials and their dispersal patterns. This is because naturally produced antimicrobials are usually present at very low concentrations, often below the detection limit, and only released locally at microscales (Larsson and Flach, 2021). Concentrations of such antibiotics would be expected to drop rapidly around the producing organisms, which results in limited exposure and further dispersal. Man-made antibiotics, on the other hand, act on macroscale levels as they disperse beyond their site of administration and in concentrations that can result in hormetic effects and selection pressures across entire microbial communities (Wright, 2010).

9.3 Selective effects of sub-lethal concentrations of antimicrobials and combined exposures

Several pharmaceutically produced antimicrobials have been tested as single compounds for activity in microbes at concentrations below those needed for cell growth inhibition. Almost all of the tested compounds exhibit biological effects (Anderson et al., 2006; Stanton et al., 2020). Thus, there is a clear difference between the sub-lethal concentration that causes selective effects (MSC) versus the concentrations that inhibit growth with bacteriostatic or bacteriocidic effects. Bengtsson-Palme and Larsson introduced the term predicted no effect concentration as a general approach to calculating the emission limits for antimicrobials in relation to their effect on resistance selection (Bengtsson-Palme and Larsson, 2016).

Bacteria in the environment will be exposed simultaneously to a range of man-made chemicals of which some are considered contaminants/pollutants. This exposure includes antimicrobial compounds, PTMs, and biocides. Exposure considerations should therefore take into account the overall concentration ranges reached by various antimicrobial classes as well as co-selection mechanisms (Seiler and Berendonk, 2012). Co-selection for AMR among bacteria exposed to biocides used as disinfectants and to PTMs (especially Cu and Zn) used as growth promoters and therapeutic agents for some livestock species are known (Wales and Davies, 2015). The fields of eco- and mixture toxicology have not yet been fully developed to address the environmental pillar of resistance (Kraemer et al., 2019; Thiele-Bruhn, 2019).

9.4 Methods for detection and quantification of antimicrobial agents in environmental samples

The availability of accurate methods for the quantification of antimicrobial compounds in the environment is important to determine the environmental footprint of antimicrobials. The ability to detect and quantify antimicrobials in complex samples will depend on the nature of the sampling matrix and the chemical structure of the antimicrobial compound itself (Yang et al., 2021).

Representative detection relies on several steps including the choice of sample sites, sample size, and the determination of the analytical limitations for the specific antimicrobials being measured. Often, a pilot study to determine the spatial and temporal scales of variation in the presence and concentration of antimicrobial agents needs to be performed before a stratified sampling regime can be designed that adequately represents the spatiotemporal variation in agent concentrations.

Given the wide range of antimicrobial agents in current use, the analytical methods used to detect them in different environmental contexts are correspondingly diverse. The methods used for detection of antimicrobials in complex samples such as soil, faeces, and manure typically include various types of solvent extraction, chromatography, and mass spectroscopy. See Box 3 for an example.

In cases where the presence of antimicrobial agents is considered in the context of parallel sampling of AMR traits, one must consider the possible separation in space and time between the current detectable antimicrobial agents and the microbial resistance patterns observed. As a hypothetical example, the patterns of AMR in *E. coli* in cow faeces may be explained by exposure to bacteria/resistance traits present in grass and water sources that are no longer available due to changing seasons. In short, careful study design is essential and must be based on a knowledge of statistical power, analytical precision, and the specific biological system being considered.

Box 3 – Detection of antimicrobial agents; example case.

As an example, we may look at a study recently performed in Norway. The study by NVI and NIBIO was published in 2021 and assessed the concentrations and prevalence of selective agents and AMR microbes in terrestrial environments. Aggregate samples of soil were taken from the top layer (0-5 cm depth), frozen shortly after sampling, and kept at –20°C until

analysis. Water content had to be addressed to obtain comparable concentrations, so parts of each sample were dried for weighing while 10 grams of each sample were extracted with acetonitrile and analyzed for pesticide content with NIBIO's screening method using LC-HRMS (Thermo LC-QExactive Orbitrap), which includes searches for 800 pesticides and metabolites together with a complementary screening database of 100 antimicrobials. The compounds detected in the soil samples were verified using mass spectrometry. Pyraclostrobin-d3 was used as the internal standard to adjust for recovery and possible matrix effects in all samples. Before analysis of heavy metals and other elements, the soil sample was dried, sieved, and mortared before being decomposed with nitric acid in an Ultrawave, diluted with water, and analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Nesse et al., 2021).

10. AMR bacteria and genes in the environment

Our understanding of bacteria in the environment is limited, and it is estimated that of the microbial diversity on Earth more than 99% of the approximately 10^{12} microbial species remain undiscovered to date and only a small fraction can be cultured by current techniques (Bodor et al., 2020). A study by Su and Wen in 2022 suggested that nearly 12% of Earth's microbial genomes carry ARGs and that the human gut microbiota is an important reservoir of ARGs. Their study found efflux-mediated multidrug resistance genes to be the most predominant ARG type and Enterobacteriaceae species to be the largest hosts of ARGs. The study identified 36 subtypes of high-risk ARGs across all ecosystem categories and ranked the importance of ARG-carrying species for representing antibiotic resistance risk based on the coexistence of ARGs, virulence factor genes, and mobile genetic elements in the bacterial genomes (Su and Wen, 2022). The authors suggested that based on ranking the importance of ARG-carrying species in the different ecosystem categories, several bacterial species such as *E. coli, E. faecalis*, and *Pseudomonas stutzeri* are recognized as priority species for surveillance and control. These bacteria may generally be found in many different niches in the environment but always in wastewater. The origin of these bacteria in the environment may be faecal contamination, zoonoses, wildlife, and agriculture.

10.1 Acquisition and transfer of resistance

In contrast to intrinsic AMR, acquired resistance is the result of spontaneous or environmentally triggered mutations and/or horizontal gene transfer events. The initial acquisition of new resistance traits usually occurs at low rates. Hence, a natural population of a bacterial species will most often contain cells that are resistant to some antibiotics, as well as cells that are susceptible to the very same agent.

Some resistance traits can arise through genomic mutations, for instance, mutations in ribosomal RNA genes can confer resistance to antibiotics that inhibit protein synthesis. The majority of resistance traits observed in the clinic are acquired through horizontal transfer of DNA fragments, including mobile genetic elements. The acquisition of resistance in bacterial populations/species occurs as discrete events separated in time and space. This heterogeneity in processes coupled with chance events can sometime result in the same resistance trait in a given species having a different genetic origin or the same resistance trait being acquired at different time points (Edwards et al., 2020). The observed current set of resistance traits in each bacterial population is thus the combined outcome of the environmental conditions, its selective conditions, the various gene transfer mechanisms and pathways, and migration.

Genetic elements that harbour resistance to antimicrobials occur in most known environments. Most transferable resistance traits are genetically linked to mobile genetic elements such as transposons, insertion sequences, and integrons. These elements can also be part of plasmids and facilitate mobility of genes within cells, e.g. from a chromosome to a

plasmid. Plasmids and integrative conjugative elements can also facilitate gene transfer between cells through conjugation (transfer of genetic material through direct contact). Other intercellular mechanisms of exchange that facilitate transfer of resistance traits are transduction (transfer of DNA from host to recipient through the actions of bacteriophages) and transformation (uptake of extracellular DNA directly from the environment). These mobility mechanisms often interact to facilitate the evolution and dissemination of AMR given environmental conditions that favour these events through selection (Liu et al., 2021). Environmental factors can also affect the rate of horizontal gene transfer, and it has been reported that stressed bacterial cells can more readily attract or accept laterally transferred genes, including ARGs (Baharoglu et al., 2013; Zhao et al., 2020; Zhu et al., 2017).

Common examples of genes encoding resistance determinants that can be spread horizontally are those encoding enzymes that modify or destroy the antimicrobial substance or that modify the antimicrobial target. For example, the AmpC β-lactamase degrades penicillin, acyltransferase enzymes render chloramphenicol inactive through acetylation of the drug, and vancomycin resistance can result from modified peptidoglycan precursors to which the drug does not bind with high affinity.

10.2 Prevalence of (transferable) resistance genes

AMR genes and organisms are ubiquitous in natural environments (Su and Wen, 2022) where they are thought to be important determinants of biotic interactions between microbial cells and populations (Davies and Davies, 2010; Hiltunen et al., 2017; Larsson and Flach, 2021). AMR predates human antibiotic use, with known resistance genes detected in 30,000-year-old permafrost (D'Costa et al., 2011) and in a cave environment that is thought to have been isolated for more than 4 million years (Bhullar et al., 2012). Reconstruction of ancient bacterial genomes from the distal gut of the mummified Tyrolean Iceman Ötzi, who lived more than 5000 years ago, revealed that antibiotic resistant human-associated species were present in the Copper Age (Lugli et al., 2017). Numerous recent studies have identified AMR genes in marine (Hatosy and Martiny, 2015), freshwater (Di Cesare et al., 2015), and soil (Forsberg et al., 2014) environments, even in more pristine environments like Arctic soil (Van Goethem et al., 2018) or the microbiome of an isolated tribe in the Amazon jungle (Clemente et al., 2015). Although AMR genes can be considered widespread, and there is a baseline of a natural resistome, it is evident that the resistance problem observed today is the outcome of an altered resistome due to human activities. The natural resistome can hence be considered as a contaminated resistome (Manaia, 2017).

The current resistome can be hypothesized to contain higher levels of ARGs that are transferred using more effective vectors and that are expressed at higher levels. While we do not yet have a clear picture of the roles that environmental resistomes play in the transmission of AMR genes and the evolution of new resistant pathogens, there is evidence suggesting that transfer among non-pathogenic and pathogenic bacteria may be frequent and can occur across significant phylogenetic distances (Forster et al., 2022). The review article of Su and Wen (Su and Wen, 2022) suggested that nearly 12% of Earth's microbial genomes are ARG carriers. However, it is likely that some external environments are

hotspots for resistance development, such as environments subjected to pharmaceutical pollution or to faecal matter containing wastewater/sludge (Pal et al., 2016).

According to Pazda et al. (Pazda et al., 2019), there is a reliable confirmation that wastewater and WWTPs are hotspots for the spread of antibiotic resistance in the environment. Furthermore, Sanchez-Baena et al. (Sánchez-Baena et al., 2021) studied ARGs in the aquatic environment and concluded that the ARGs that are mainly reported in the urban areas of the world are those that confer resistance to the antibiotics that are most used in clinical practice, which constitutes a problem for human and animal health. Yang et al. (Yang et al., 2022) identified 1926 unique types of ARGs and discovered that the ARGs are more abundant and diverse in the mesopelagic zone than other water layers. They also found that ARG-enriched genera were often more abundant compared to their ARG-less neighbours in the same environment (e.g. coastal oceans).

10.3 Persistence and reversal/loss of acquired resistance

Acquired AMR is subject to selection due to its effect on host fitness. The persistence of acquired resistance traits in a microbial population is thus a function of the benefits bestowed by protection against antimicrobials and the fitness cost associated with producing additional enzymes or altered structural biomolecules. Mutations that impose little or no fitness cost can persist in the population even in the absence of positive selection exerted by the presence of the antimicrobials in the environment (Kassen and Bataillon, 2006). However, acquired AMR can put the host strain at a competitive disadvantage relative to susceptible strains in the absence of selective agents. For example, resistance may entail reduced cellular mobility (Stickland et al., 2010) or reduced ribosomal function (Holberger and Hayes, 2009).

In cases where acquired AMR has not been found to incur a significant fitness cost, it has been linked with co-selection of resistance genes and fitness-conferring loci (Borrell et al., 2013) and with compensatory mutations in secondary sites that increase fitness without affecting resistance (Comas et al., 2012). There are also examples where single mutation-based resistant strains do not show reduced fitness relative to wild type bacteria or even increased fitness (Kassen and Bataillon, 2006). An extensive meta-study found that most resistance mutations entail a fitness cost to bacteria and that the degree of resistance (increased MIC) is inversely proportional to the fitness of resistant mutants (Melnyk et al., 2015).

These studies suggest that many, but not all, acquired AMR is dependent on a selective agent to persist in a population over time, and by extension in a microbial community. In an environmental context, this means that the selective conditions for AMR persistence can be found in polluted environments associated with sewage treatment or intensive agriculture. As discussed above, studies have found that antimicrobials have selective effects far below MIC values (Gullberg et al., 2014) and selection of resistant mutants can occur at concentrations comparable to those found in the environment (Stanton et al., 2020). Most studies of fitness costs, selection, and persistence of AMR have been done in laboratory culture experiments,

usually involving a single species, and less is known about the dynamics of AMR genes in the environment. It is noted that methodological limitations prevent measurements of minor changes in fitness costs in bacteria in laboratory settings.

It is likely that the selection on AMR mutants in situ will be influenced by other biotic interactions that occur in complex communities. For example, a susceptible bacterium can be protected through extracellular inactivation of the antimicrobial substance by other community members (Yurtsev et al., 2013). A recent study (Klümper et al., 2019) found that resistant *E. coli* showed a much higher MSC when growing as part of a complex community as compared to when grown as single species cultures, demonstrating potential discrepancies between traditional fitness assays and selection in the environment (Anderson et al., 2006; Kraemer et al., 2019; Stanton et al., 2020).

11. Interfaces of agents, genes, and microbes

A key hazard driving environmental surveillance is the potential for and adverse outcomes of AMR transferring from environmental sources to clinical settings. Hence, it is important to understand the interfaces/exposure sites where genes, selective compounds, and bacteria meet. These are sites where resistant bacteria and resistance traits evolve in a particular environment may transfer in a directional manner to the new environmental conditions. In addition to a linear model, bacteria or resistance traits may cycle between environments with variable vehicles, time intervals, and spatial routes.

Natural vehicles for dissemination of microbes between environments and ecosystems include water and air currents, as well as the mobility of all kinds of biomass, including different animal species and humans. Anthropogenic activities, such as WWTPs and agriculture release large numbers of microbes, often in combination with environmental contaminants, in the form of effluent water, sludge, and manure. High levels of international travel, transport, and trade of live animals, food, living plants, and organic fertilizers result in AMR dissemination routes of a global scale. Furthermore, international supply and transport chains have the potential to translocate vast numbers of microbes between remote locations, e.g. through the release of ballast water or when present in commodity crops. Thus, the potential for international spread of resistance genes and microbes is high and is probably increasing.

Figure 1 illustrates the many interfaces that bridge the human, animal, and environmental pillars of One Health. These interfaces are also considered key arenas for further investigation because they define the major one-dimensional or multidimensional pathways for the dissemination or spread of antimicrobial agents, AMR genes, or microbes. Increased understanding of these pathways, the identification of transmission chains, and opportunities for interventions should be key goals in environmental surveillance. A longitudinal design of monitoring efforts is also necessary to understand the temporal and spatial characteristics of the pathways of resistance flow inherent to different interfaces. Some important interfaces include the following.

Wastewater, sludge, and manure Wastewater effluents, sludge, and manure-fertilized soil systems have been identified as key interfaces for AMR in a One Health perspective. The effluents released from WWTPs are considered to have a central role in AMR dispersal to the environment. The scale of operations suggests significant release rates of both antimicrobial agents, AMR genes, and microbes (Singh et al., 2019). Aquatic systems link these compartments, with animal and human exposures completing the dissemination circle of AMR genes and bacteria between the three pillars. For instance, when resistant bacteria and resistance genes are distributed with wastewater sludge or with animal manure, they reach arable land when the sludge and manure are used as soil improvers and fertilizers. They may thus be recycled into the food-production chain. Through this cycle, resistant bacteria and

ARGs will be introduced into new environmental compartments to which they adapt and to microbial communities in which they must compete for survival and growth. Depending on the bacterial species, these new environmental compartments will most likely be hostile, but they will also provide opportunities for microbial interactions, including horizontal transfer of ARGs within and between bacterial species.

Biofilms In most environmental settings, microbes live in biofilms. Biofilms are complex multispecies microbial communities attached to abiotic or biotic surfaces and with suitable conditions for horizontal gene exchange. A study of freshwater biofilms revealed that these biofilms were reservoirs of ARGs with high richness and abundance, and that geographical location and human footprint significantly affected the resistome in the freshwater biofilm samples (Yao et al., 2022). Several of the identified ARGs were associated with mobile genetic elements and were found to occur in both known pathogens and human gut bacteria.

Food sources Food of all origins represents an important interface linking microbes from plant and animal reservoirs as well as open and processing environments with workers along the food chains, food dealers, and consumers. As described above, the NORM-VET system surveys AMR patterns of faecal indicator bacteria isolated from Norwegian food-producing animals. However, several data gaps regarding AMR in the food chains were identified in the VKM report "Assessment of antimicrobial resistance in the food chains in Norway" (VKM et al., 2015)

Recreational water Marine and freshwater have environmental microbiota that are impacted by characteristics and activities in the catchment areas. When these waters are used for recreational purposes, like swimming and other water-associated activities, humans will be directly exposed to the resident microbes (Leonard et al., 2015; Maravić et al., 2015). There are some studies that confirm the presence of ESBLs in recreational waters, although the fraction of ESBL compared to the number of general *E. coli* has been low (Jørgensen et al., 2017). Urban runoff water has been identified as a hotspot for AMR spread (Almakki et al., 2019).

Animal migration Some wild animal species are, from time to time, included in the NORM-VET report, but the data retrieved are limited. However, wildlife may be regarded both as a reservoir as well as vector for the dissemination of AMR. A previous report from VKM (VKM et al., 2018) examined AMR in wildlife and concluded that animals, particularly migratory birds, are considered to have a broad dispersal capacity for AMR bacteria. It was concluded that "wildlife species moving over long distances provide a link between different areas and environments, and consequently represent a potential for transport and introduction of AMR genes and bacteria to food chains or humans. Such introduction could be both direct and indirect, as the long-distance migratory species could transmit resistance genes and bacteria to wildlife living closer to humans. VKM considered important species in Norway of particular concern to be geese, swans, and ducks that may overwinter in areas with high human population densities or high livestock densities, and subsequently visit lakes, agricultural land, and parks in Norway. Another concern expressed by the VKM was the possible role of long-distance migratory seabirds that are in contact with gulls, which could contaminate urban and agricultural environments with AMR bacteria" (VKM et al., 2018).

In conclusion, important interfaces and environmental matrices that may be considered further for sampling include aquatic systems such as sewage, rivers, drinking water, and marine environments. Water is also a vehicle for the transport of microbes (including antimicrobial resistant microbes) from one location to another. Other interactions can be more local regarding the exchange of microbes (e.g. fertilized farmland, feeding places for grazing domestic and wild animals, and feeding or hatching places for migrating birds).

12. Assessment of opportunities and limitations of a surveillance system

Many initiatives have emerged that explore if and how the environmental dimension of resistance can be included in One Health-oriented AMR surveillance. As exemplified in chapter 4, these include initiatives by UN institutions such as the WHO, UNEP, and FAO as well as the EU. Ongoing research efforts in the scientific community have informed the above-mentioned initiatives by calling out the many knowledge gaps in our current understanding of the environmental pillar of resistance. The many international initiatives, publicly supported research programmes, and the scientific literature emphasize the need to strengthen the One Health approach and increase our efforts to understand the environmental dimensions of resistance, including potential risk to human and animal health.

Here we have explored the opportunities and limitations of the approaches to environmental AMR surveillance by considering the available scientific literature. Several key findings have emerged from our work. This includes the observation that calls for surveillance are not uniform and straightforward to implement. Moreover, the rationale for environmental surveillance differs from clinical surveillance of AMR, and the methodology, tools, and techniques used to determine environmental resistance lack the level of standardization available in clinical settings. It is also clear that environmental populations of bacteria are considerably more diverse than the set of bacterial pathogens known to cause disease in humans and animals. These large differences in bacterial diversity and their heterogeneous environments exclude direct adoption of clinical sampling approaches to environmental settings. Hence, as further discussed below, an environmental surveillance approach requires careful consideration of achievable goals, contextualization with other surveillance efforts, prioritization of the most relevant target populations, sites and time intervals, and the refinement of sampling methodology and the standardization of data formats for comparative purposes. In this context, the scientific literature also highlights opportunities to draw on existing sampling efforts to reduce costs and to narrow the bacterial diversity considered to well-characterized species also occurring in clinical/anthropogenic contexts, for instance, the gut bacterium *E. coli*.

It is noted that the important concept of "surveillance" is not uniformly understood. For some applications, "surveillance" assumes the activity of maintaining an oversight of identified hazards and collecting data that can inform risk assessment and policy development and can trigger interventions, for instance, to respond to changes in the prevalence of multi-drug resistant clinical pathogens and recommend changes in drug therapy guidelines. In environmental settings, the word "surveillance" is also used somewhat synonymously with "monitoring" where the latter indicates a more neutral stance to how to respond to repeated observations over time. Such "monitoring" describes the activity of systematic data collection over time seeking to strengthen a generally weak knowledge base rather than supporting interventions. Most of the rationales of environmental "surveillance" fall into the latter purpose, namely the data collection and knowledge building that may, at a

later yet unspecified stage, be used in combination with clinical surveillance data to inform policy and interventions. Below we present some of the key premises, opportunities, and limitations of the surveillance of AMR in the environment.

12.1 Key premises behind a surveillance system

The rationale for surveillance is clearly defined

A key finding is that the rationale for environmental surveillance will differ from that of current, clinically oriented AMR surveillance programmes. This difference in orientation has several explanations. First, current clinical surveillance efforts focus on well-characterized species of pathogenic bacteria with known resistance profiles and disease transmission pathways. Second, tools for the isolation and identification of these single-species of pathogenic bacteria have been developed together with standardized protocols for resistance characterization in the context of therapeutic success. Observations of changing susceptibility patterns in the studied pathogens can be considered against clinical breakpoints and can inform best practice, motivate interventions, and improve clinical drug prescription guidelines. These means and work practices have been standardized at the international level over decades.

The practices developed in clinical settings thus cannot be directly applied to understanding the resistance dynamics between microbes present in diverse, open environments. This is also due both to large differences in the biology of the systems considered as well as the fact that resistance is defined in the context of a specific host and therapeutic treatment.

As described in more detail in chapter 3, the rationales for surveillance of environmental populations of bacteria can be described as the need to develop:

- A better understanding and control of hazards/risks to human and animal health of/from the environment as a source/sink for AMR development, persistence, and transfer
- A holistic understanding of ecosystem health supported by data-driven analyses and intersectoral surveillance approaches at the international level
- Proactive approaches to support reduction of AMR and antimicrobial agents in the environment (based on the precautionary principle and acknowledging complexity, knowledge gaps, and uncertainty)
- An understanding of the biological effects of dispersed antimicrobials as pollutants in the environment
- Applied approaches to estimate antimicrobial usage and resistance levels (including in humans, animals) through indirect data collections

Moreover, specific environmental surveillance efforts may be called for through international obligations and policy developments responding to the above considerations.

Considering the variable rationales, it is imperative that an environmental surveillance effort is transparent and clear about its goals and deliverables. It appears from the scientific

literature that deliberations over the merits and goals of environmental surveillance of AMR are not yet concluded.

The specific knowledge gaps addressed with the particular surveillance effort are clearly stated

From the considerations of the identified rationales (chapter 3), it is clear that the various emerging surveillance initiatives will address different knowledge gaps and will have different deliverables. An important rationale behind many surveillance efforts is to reduce the knowledge gaps in our understanding of environmental resistance, particularly at the interfaces where human, animal, and environmental microbial populations meet. The data collected will ideally reduce several gaps present in our current fragmented understanding of the emergence, persistence, and transferability of resistance genes and microbes, as well as the effects of resistance-driving antimicrobials and other chemical compounds. Environmental surveillance efforts must therefore be transparent in their aims, objectives, endpoints, and deliverables towards the knowledge gaps addressed. A well-developed communication plan is needed that considers all relevant user groups of the collected data. This includes communicating the specific knowledge gaps the particular surveillance effort reduces and how the surveillance effort adheres to, builds on, and is contextualized to other national and international efforts.

The surveillance effort is transparent with regard to the description of uncertainty and risk

Ideally, new surveillance-based data will support research-driven efforts that can, in the long-run, build robust biological models with causal relationships that support hazard identification and risk assessment. A classic definition of risk is (risk = likelihood × hazard). The likelihood part of the risk equation is a core challenge in environmental risk assessment. The limited opportunity to determine numeric likelihood is due to, among other factors, the limited understanding of the components of the hazard as such, the relevant time frames, system complexity and the scale of the environment considered. Moreover, a specific risk may be accumulative and may not be fully addressed through the risk/environmental scenarios considered and its corresponding sampling schemes (model uncertainty). The work of the EFSA (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5123 and https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5122) on uncertainty analyses in scientific risk assessment should be consulted when further developing surveillance efforts.

Surveillance efforts are likely to reduce some knowledge gaps as well as identify new ones. Nevertheless, strengthening the knowledge base will reduce the overall uncertainty related to human impact on the environment. Ideally, longitudinal surveillance data will increase our capability to predict how resistance patterns will change in the future. Data and new insights will support further development of mathematical models, including artificial intelligence-based approaches, that can assist in the prediction of future changes in resistance dynamics.

The surveillance effort is based on resource-effective prioritization

An almost infinite number of environments/microbial populations/chemical compounds can be placed under environmental surveillance. Hence, the approach selected will be the result of deliberations among various scientists and stakeholders. Such considerations also depend on funding mechanisms and the user groups of the surveillance data. Various types of implicit cost/benefit analyses as well as adherence to international initiatives will also affect if, and if so how, such surveillance efforts will materialize at the local, national, and international level.

The limited availability of resources must be addressed in all circumstances. Hence, opportunities to expand on existing surveillance efforts must be carefully considered for sustainable long-term efforts. The baseline surveillance should be complemented with additional targeted approaches as needed. This is also currently done in the NORM/NORM-VET system.

The process of identifying if, and if so how, a resource-effective surveillance system for environmental ARM could be designed and implemented includes a range of interested parties/stakeholders:

- Scientists/research networks
- Clinicians/veterinarians
- Public health authorities
- Food authorities
- Current set of surveillance systems funders/drivers
- Citizens/Non-governmental organisations
- Farmers and the commercial agri-food chain

These stakeholders are relevant both at the local, national, and international level, including neighbouring Nordic countries with similar environmental conditions, the EU, and globally through health and environmental organizations such as the WHO, FAO, and UNEP.

The most risk-relevant targets/sites/conditions have been identified

To enable prioritization, hazards must be identified and the most risk-relevant targets/sites/conditions ranked. Available methods to close or reduce corresponding knowledge gaps can be identified and considered in the context of other efforts at the international level.

Further deliberations among scientists and the various stakeholders are likely to reveal the most relevant environmental matrices, target populations, and sites according to current knowledge. These choices will vary with geographical location and with the level of anthropogenic influence and level of pollutants.

The basic analytes are microbes, genes (DNA), and selective compounds, as discussed in more detail in chapter 8. Current experimental studies of environmental AMR represent variations on how to approach these analytes as exemplified below.

- Microbes

    - Longitudinal monitoring of resistance patterns of defined, culturable microbes with known human or veterinary pathogenicity.
        - E.g. resistance phenotypes of ESKAPE pathogens and enterobacteria recovered from environmental sites/hosts
- DNA
    - Longitudinal monitoring of the metagenomic composition of environments to identify changes in resistance gene patterns over time.
        - E.g. metagenomic profiling of agricultural soil after the use of organic fertilizer
- Selective compounds/chemicals
    - Longitudinal monitoring of antimicrobials (as pollutants) in various environments as possible drivers of resistance development.
        - E.g. measuring the concentrations of antimicrobials in the sediments or grounds of (fish) farms.

One Health-guided AMR surveillance efforts should consider all three analytes in combination with defined environmental matrices. For the DNA analyte, information may simultaneously be obtained for both the known resistance traits as well as the microbial diversity present in the sample. Depending on the methods available, resistance traits may be linked to the host microbe. DNA is likely to be the main analyte in the foreseeable future given that most environmental microbes cannot be grown in the laboratory and DNA-containing samples may be more easily obtained from combined sampling efforts.

As considered above, a basic premise for a sustainable surveillance effort is resource-effectiveness. This will necessarily limit the number of interfaces, microbes, and microbiomes and the resistance traits, genes, and selective compounds considered for surveillance efforts.

Three approaches to resource-effective design include narrowing the focus to i) established indicator species such as *E. coli* with environmental counterparts or cyclic lifestyles that are well-characterized, ii) a defined set of important resistance genes through PCR or WGS, or iii) metagenomic analyses of sewage for continuous global surveillance.

Resource-effectiveness can also be achieved if resistance patterns in a subset of analytes present in each environment are considered to be representative of the larger system. For instance, the determination of the prevalence and composition of resistance patterns in some bacterial species (e.g. environmental pathogens) would be indicative of the overall resistance level, or the determination of the prevalence and composition of integrons would be indicative of the overall resistance level and level of anthropogenic influence.

Another possibility to prioritize based on known risk is to focus on those microbes that are rapidly developing AMR, namely the ESKAPE group of pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacte*r spp.). Several of these species can also be recovered from various environmental sites.

The prioritization efforts, as exemplified above, may skew the selection of analytes to those with an established methodological framework, for instance, to the detection of the currently most common resistance genes or bacterial species known to cause resistance/disease in humans and animals. This may help reduce complexity and facilitate integration with other surveillance efforts. On the other hand, environmental surveillance may also concern novel resistance traits and environmental and emerging pathogens that are currently not part of clinically-oriented surveillance programmes. In all cases, communication of the prioritization made should make explicit the assumptions and trade-offs made in the formulation of objectives and selection of analytes.

Untargeted metagenomic approaches, e.g. looking at how community-level resistance patterns change over time, is also conceivable. The outcome of such approaches will depend on the sequencing technology and the resistance databases used and may not yet be suitable for a global uptake. Broad international uptake of surveillance is a necessity for generating data that can build understanding of complex resistance dynamics materializing at the global level. Collective study, data sharing, and comparison and interpretation of resistance data will be the foundation for successful surveillance efforts.

The surveillance effort is an integrated part of a One Health framework

As considered above, single environmental surveillance approaches are unlikely to drive interventions and support robust policy formation. It is therefore of great importance that the environmental surveillance data be collected in a form that allows integration with other environmental or clinical surveillance data. The resource and knowledge-building effectiveness of a new environmental surveillance programme will be defined by how well-anchored, integrated, and positioned it is to the current knowledge base and the existing clinically oriented AMR surveillance and other environmental programmes. For the latter, see examples in Appendix 3.

Many current environmental programmes focus on chemical compounds unintentionally present in the environment and that may cause direct or indirect harm. These are defined as pollutants. Although it is clear that antimicrobials in the environment are pollutants, the extent to which DNA and resistance genes can be considered pollutants in the environment is still a matter of debate (Woegerbauer et al., 2015) because their presence in the environment does not cause a direct harm. The risk from such substances emerges from the indirect effects caused by horizontal transfer and subsequent amplification of the trait in pathogenic bacteria. The various international initiatives, as also presented in the Appendices, suggest increased focus on antimicrobials as pollutants.

The surveillance data are collected through standardized methods and reporting, taking into account natural variation

A key insight from our systematic review is that current approaches to environmental resistance are limited by fragmentation and lack of standardization. Fragmentation is due to the often-arbitrary choice of the environmental sites considered, the specific sample sites, and the sample numbers and time points. Very few studies include time series or attempt to

describe resistance dynamics over time. Most studies are observational and report on point prevalence. Statistical considerations beyond technical variation are rarely made. Few studies attempt to describe the natural variation inherent to the matrix studied, e.g. seasonal dynamics or how sampling limitations/artefacts of external factors contribute to the point prevalence observed.

The analytes as well as their data reporting formats vary between environmental resistance studies. This variation in scientific practices limits and often prevents systematic, comparative, and longitudinal analyses. Because of the above, the knowledge base of environmental resistance appears as a collection of studies, with limited direction or coherence.

In contrast, clinical approaches have worked on reducing these limitations through decades of efforts through EUCAST, etc. To reduce further fragmentation and to start building a collective database, environmental surveillance efforts must before onset work out the terminology used, the sampling design, and the analytical methods taking into account both natural and technical variation. Moreover, a data storage platform must be developed that allows for identification of and access to data in a uniform format in order to allow reuse of data.

The complexity of the environmental dimension is acknowledged

It is important to recognize the high complexity of the environmental dimension of resistance. A reductionistic/mechanically oriented expectation of surveillance programmes to identify causalities, pathways, and processes that will trigger specific interventions is not likely to be met soon. Acknowledging complexity triggers different dialogues among stakeholders about what can be achieved through the systematic collection of environmental data over time. The acknowledgement of complexity refocuses the rationale to one of strengthening a generally weak knowledge base. One view will be that more systematic, standardized, and internationally coordinated efforts will be more efficient at unfolding the complexity of resistance biology and its drivers compared to sporadic local initiatives. Moreover, such systematic approaches are needed to strengthen One Health-based interventions in the long run. The opposite view could be that the biology of environmental AMR is too complex to address with surveillance or that the outcome is unlikely to inform interventions.

12.2 Opportunities offered

Given that the above premises are met, a Norwegian initiative on environmental surveillance offers many opportunities:

Systematic and coordinated efforts to build knowledge across environments that will close knowledge gaps and reduce uncertainty

Environmental surveillance will collect data, build competence, support data infrastructures, and contribute to research advancing the biological knowledge of environmental resistance, including anthropogenic effects.

Support and contribute to international initiatives on environmental resistance

Contribute to further development of WHO, UNEP, FAO, and EU initiatives. Particularly in the EU, contribute to the surveillance of AMR and antibiotics and their residues within the environment and to the Strategy on Pharmaceuticals in the environment, the revised EU AMR action plan, and the Green Deal initiative.

Strengthening the basis for the National Strategy against AMR

- Contribute to the Norwegian Ministry of Health and Care Services National Strategy against Antibiotic Resistance (2015-2020. Publication number I-1164 E) stating "the AMR problem is complex and there is still a lack of knowledge before we have a holistic understanding of natural and man-made factors that prevent or promote the development of resistance" and further development of a data-driven national strategy.

Establish a One Health orientation towards AMR in Norway

- Collect complementary data to the established clinical (NORM) and veterinary (NORM-VET) surveillance programmes in Norway. Given some overlap in the identification of the most risk-relevant targets, sites, conditions, a "NORM-ECO" surveillance programme can complement the clinical and veterinary clinical data with data obtained from prioritized environmental matrices. This will support a One Health orientation to resistance.

Contribute at an early phase to develop methodology and standards

- Various surveillance initiatives are now considered. Early engagement and leadership from the Norwegian side could help drive the development of programmes that are sustainable, coordinated, and oriented to the most pressing knowledge gaps. Moreover, national efforts can contribute to the development of terminology and developments towards "Open Science" – ensuring that data collected through the programmes are identifiable and available at the global level.

Develop a resource-effective approach by drawing on existing surveillance/sampling efforts

- The various existing surveillance programmes in Norway offer the opportunity to focus on cost-efficient integration of an environmental AMR surveillance programme. Such a programme can benefit from existing standardized surveillance methodology and sampling systems (NORM and NORM-VET) as well as sampling routines developed for other pollution-oriented environmental programmes.

Taking a stepwise and scalable approach to a global health problem

- A Norwegian surveillance programme must inform, be informed by, and be contextualized with programmes now emerging at the international level. Hence, a programme can be developed in a step-wise manner, focusing on the most pressing knowledge gaps in Norway and at the same time responding to expectations of data collection and presentation emerging at the international level.

12.3 Inherent limitations

As discussed throughout the report, there are many yet unresolved limitations inherent in the approaches to environmental surveillance

Complexity of the biological systems considered

The biological systems considered are composed of intricate networks and mechanisms of i) the dispersal of organisms, including means of migration, ii) the units, vectors, and pathways of resistance gene flow, and iii) the release, dispersal, and decay of compounds. The complexity of these systems and their variation over time and space severely limits the representativeness of any sampling-based surveillance effort. The biological complexity entails many unknowns and interrelated factors behind the multiple risk scenarios considered.

Causality can rarely be established in intricate biological systems

Molecular epidemiology is used to establish recent origin and transmission pathways of bacteria (e.g. outbreaks). This is facilitated by the use of multiple genetic markers. For mobile resistance genes, fewer markers are available, and it will often not be biologically possible to trace the specific evolutionary pathways taken by a given resistance trait. Hence, methods to establish directionality of gene flow, or uncontested "evidence" for the resistance trait to be the cause of a particular exposure/sources is often not obtainable. The important statement of "Absence of evidence is not evidence of absence" (Alderson, 2004) is valid in this context too. The implicit call for the need to establish "evidence" to show causality is distracting a scholarly debate, and in most cases is futile for a given resistance trait. Hence, it is important to acknowledge that causation cannot be the goal of current environmental surveillance, in contrast to the opportunity to establish a correlation.

Hence, most surveillance efforts will, for the time being, focus on strengthening the knowledge base through data collection, analyses, and reporting with a long-term goal to understand causality and directionality of resistance flow between environments.

Limited opportunity to intervene

Clinical resistance data offer opportunities to intervene according to the specific pathogen and antimicrobial treatment regime. Corresponding data on the use of antimicrobials may lead to action to change usage patterns and volumes. The opportunity to intervene based on environmental resistance patterns appears less straightforward. At the national level, it seems unclear how an observed change in a particular environmental resistance pattern

would lead to an intervention/consequence beyond what is already embedded in the national action plan. Similarly, changes in antimicrobial concentrations in the environment may not necessarily point to any specific intervention. This is because the relative contribution from the different usages/sources that drain into the environment and the relevance of interventions applied to them are often not well known.

Unclear applicability of the precautionary principle

The precautionary principle has been introduced into environmental legislation particularly in the EU
https://www.europarl.europa.eu/RegData/etudes/IDAN/2015/573876/EPRS_IDA(2015)573876_EN.pdf.

It suggests action may be taken when there is potential for harm, even in the absence of complete evidence. Actions could for instance include implementing measurements to reduce exposure from wastewater treatment plants, and to ensure a high degree of manure composting etc., despite lacking evidence of causality or full effect. Although a useful policy and risk management-oriented principle and approach, its applicability to address uncertainty in the environmental dimensions of resistance remains to be fully determined. The environmental surveillance initiatives can find support in the precautionary principle to reduce risk-relevant uncertainty and by informing policy formation.

Approaches to cost/benefit analyses will differ among stakeholders

As discussed above, the limitations in available research methodology usually prevent establishing causality and hence the quantification of risks. This lack of a quantitative understanding of the biological systems considered, combined with complex scenarios and multiple risk scenarios, hinders the development of a cost/benefit analysis. This is because risk perceptions, scenario uncertainty, and benefits will differ among stakeholders.

Plurality of initiatives and lack of a single coordinated effort at the international level

A key limitation to deciding on, and in the establishment of, a surveillance programme is the many initiatives now being explored in parallel and that are often inconclusive at this stage. This complicates the opportunities for programme development based on the insight from well-established clinically oriented surveillance programmes as well as other environmental surveillance programmes in Norway. This creates policy scenario uncertainty at several levels. Importantly, national initiatives should be developed to adhere to international initiatives, while at the same time they should be resource-efficient and complement other national surveillance programmes. Moreover, rapid methodological developments will challenge the harmonization of approaches, which is needed for comparative and longitudinal analyses. There is also a need for the development of a common data-sharing platform that allows fair access and metanalyses of data. Because environmental resistance is a global problem, it is clear that international approaches have to include cost-efficient approaches in countries currently lacking the capacity for environmental monitoring.

12.4 Perspectives on a possible "NORM-ECO" surveillance system in Norway

A range of considerations must be made when designing a surveillance system. The rationale must be clearly established, and the most risk-relevant scenarios and knowledge gaps identified. These processes will provide the basis for the specific choice of target analytes, the environmental matrix, the detailed sampling design including spatial and temporal aspects, and how the methodological limitations of the chosen approach are accounted for.

Environmental sites

For the environmental matrix, aquatic systems such as sewage, rivers, irrigation and drinking water, and some ocean sites represent important compartments and interfaces where microbes, genes, and compounds that originated from different sources meet. Water is also the major vehicle for the transport of microbes and pollutants from one location to another. Other important compartments and interfaces, as discussed in chapter 10 and elsewhere, include farmland and feeding places for grazing domestic and wild animals, including feeding or hatching places for migrating birds.

Sampling and analysis of these matrices may be combined with existing systems for surveillance in Norway. For example, NIVA already monitors biological, physical, and chemical parameters in watercourses and coastal areas, and many municipalities have systems in place for determining microbial quality in recreational/swimming water. According to the Quality System in Agriculture, Norwegian farmers using natural waters for irrigation are obliged to analyse at least one water sample per season for *E. coli.* For ocean water, systems for surveillance linked to pollutants from industrial activities such as activity on and offshore, or fish farming, can be expanded. The Institute of Marine Research is annually surveying blue mussels (*Mytilus edulis)* for faecal indicator organisms such as *E. coli*. In 2018, the Institute of Marine Research published a report on AMR in *E. coli* isolated from blue mussels and reported several cases of resistance. Of the 75 bacteria examined, 65% were resistant to fewer than three different antibiotics, while 33% were resistant to three antibiotics from three different classes and were thus defined as multi-resistant (Svanevik et al., 2018). See Figure 4 for a proposed tiered approach and Appendix 3 for other monitoring efforts.

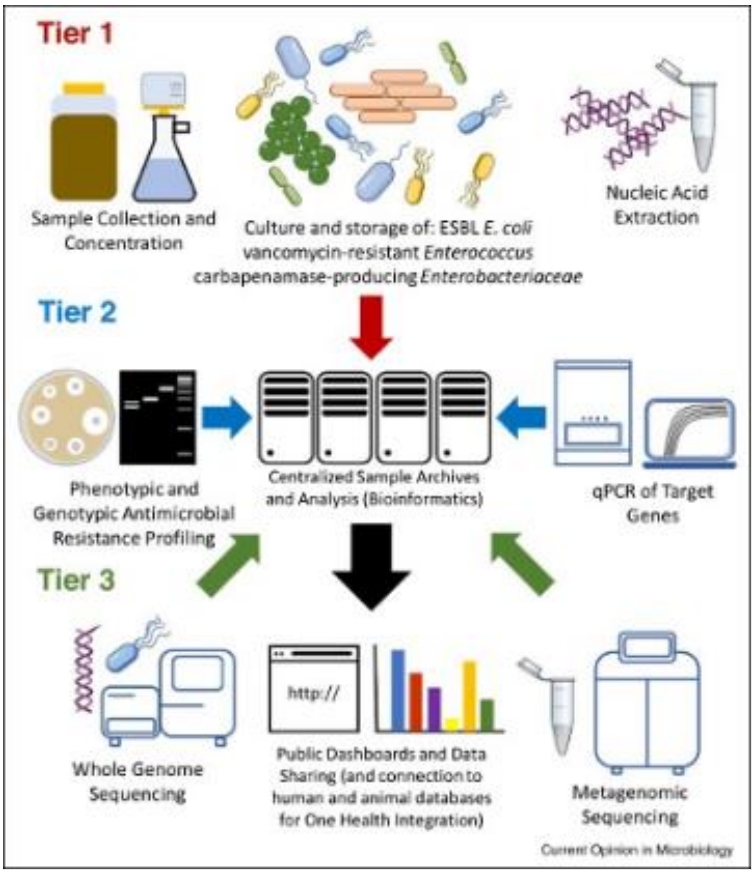


Figure 4. A tiered approach to coordinated and standardized environmental AMR monitoring. Tier 1 is most accessible and should be carried out by all participating locales. Tier 2 may be carried out in-house or by centralized facilities. Tier 3 is likely to be carried out by centralized facilities and will be least accessible due to cost, but cost and accessibility is expected to improve in coming decades. Centralized sample archives and public-facing dashboards facilitate standardization of data analysis and reporting and data sharing. Sample archives will further provide value for future re-analysis as technology evolves (Pruden et al., 2021).

The choice of environmental matrix will necessarily depend on the purpose of the surveillance. For instance, sampling of AMR in untreated wastewater upstream of treatment plants will give data on which ARBs/ARGs are circulating in society. On the other hand, such data are in general not suitable for quantitative monitoring of which ARBs/ARGs enter the environment and food chains. Metagenomic-based surveillance of untreated wastewater could be used as an early warning system for the discovery of new and emerging ARGs/ARBs. For example, when a new antimicrobial is released for use, the appearance of their corresponding resistance should be added to a surveillance panel. Such an early warning system using untreated wastewater could be integrated with NORM. In addition, sampling untreated wastewater at local levels might be relevant for assessing the transmission of AMR if there is no wastewater treatment downstream of the sampling point. This may be the case even in Norway, especially in situations with sewage overflow. Overload of sewage infrastructures has become a more relevant issue due to climate change, as we see more episodes of heavy rainfall with sewage overflow. Therefore, surveillance of

effluent water and sludge from WWTPs should be considered if a programme aims to obtain knowledge about which ARGs/ARBs are released into the environment with a potential risk for being circulated back to the food chain. Analysis of bacteria from irrigation water will be particularly interesting when this water is applied as "surface watering" on leafy greens and berries being distributed fresh and eaten without heat treatment.

Animal manure, either fresh or composted, is used as fertilizer in both grazing and crop land. Data on the occurrence of AMR in animal manure can be extracted from NORM-VET, but there is still little knowledge about the destiny of ARBs originating from the faecal microbiota being transferred to soil and through agricultural run-off, as well as the potential for dissemination of the ARGs these bacteria contain.

Methodological aspects

There are sampling methods available for environments like water, soil, and manure, and the details of the procedures will depend on the type of downstream analysis for which the samples are intended. For culturing, it is clearly important that samples include microbial communities that are as intact as possible, with viable cells that can be analyzed in live cultures. For a metagenomic approach it is usually desirable to have samples that represent, as closely as possible, the communities at the time of sampling. Instant freezing of samples can achieve this while at the same time preserving viable cells. In a field setting, however, freezing is rarely an option, and in such cases, samples are usually preserved by the addition of a bacteriostatic storage medium like ethanol. This helps preserve the original community composition in samples, but it precludes subsequent culturing. A "NORM-ECO" type of programme would necessarily entail field sampling, presumably focusing on water samples and soil or other terrestrial sites.

The methodological details of an analysis pipeline will be determined by the objectives of the surveillance programme. If the purpose is monitoring of AMR in human and animal pathogens in the environment, then a culture-based approach would be appropriate, possibly coupled with WGS and analysis of isolates of special interest. If the purpose is monitoring broader community AMR patterns in the environment, then a metagenomic approach or a multi-marker PCR approach might be more appropriate. Metagenomics is currently the method that can provide the most complete picture of the AMR gene repertoire in complex environmental samples. However, this approach requires considerable resources, both economically and in terms of technical expertise in downstream analysis of DNA sequence data. Also, this approach deviates from the established surveillance programmes implemented in NORM and NORM-VET. There is, however, a potential for automation of considerable parts of the analysis pipeline. Both for DNA extraction and sequencing library preparation much of the laboratory work can be done by robots, and bulk processing of samples can cut costs considerably. Streamlining of computational pipelines for data processing also has potential for cutting down on the hands-on workload.

Alignment with international initiatives

Comparative analyses studies from different nationally and locally-oriented initiatives are currently severely hampered by differences in purpose, study design, methodology, and parameters. A NORM-ECO initiative must therefore enable and support comparative analyses and sharing of data, taking into account the current developments in the field. The need for uniform reporting formats is addressed in the recent EMBRACE-WATERS statement: "Shortcomings exist in terms of consistent, complete, and transparent reporting in many environmental studies. Standardized reporting will improve the quality of scientific papers, enable meta-analyses and enhance communication among different experts." The authors proposed a checklist with guidance on how to report data to ensure transparent and comprehensive reporting of studies (Hassoun-Kheir et al., 2020) and One Health 2021: https://www.sciencedirect.com/science/article/pii/S2352771421001294?via%3Dihub).

The WHO Global AMR Surveillance System (GLASS: https://www.who.int/initiatives/glass) and the World Organization of Animal Health's (OIE, https://www.oie.int/en/what-we-do/global-initiatives/antimicrobial-resistance/) already provide guidance for establishing and harmonizing AMR and antimicrobial use surveillance in humans and animals, respectively. Furthermore, a recent report from UNEP (https://www.unep.org/explore-topics/water/what-we-do/monitoring-water-quality) provides data sources and exposure pathways for AMR and antimicrobial use in the environment, which can be used for integrating environmental surveillance into the existing WHO and OIE systems.

The ongoing EU Joint Programming Initiative-funded project EMBARK; Establishing a Monitoring Baseline for Antimicrobial Resistance in Key Environments (https://antimicrobialresistance.eu/) has in its mission to *"establish a baseline for how common resistance is in the environment and what resistance types that can be expected where" and to "standardize different methods for resistance surveillance and identify high-priority target that should be used for efficient monitoring"*. In addition, EMBARK will develop and evaluate methods to detect new resistance factors and thereby provide an early-warning system for emerging resistance threats.

A "NORM-ECO" surveillance system should build on these initiatives and contribute to their further development. Table 3 outlines important current surveillance activities of different environmental matrices at the international level. These will be important for further development of a NORM-ECO initiative and its responsible institutions and priorities in Norway.

Table 3. Key characteristics and the suggested Norwegian setting and priority of international projects and programmes (partly subtracted from Annex II and III) of possible relevance to environmental AMR surveillance.

Main matrix	Sample site	International setting	Microbiology target	Method	Goal	Proposed responsible institution in Norway	Suggested priority
Drinking water	DWTP	**WHO One Health survey** (Appendix AII.2)	ESBL-*E. coli* (concentration, ratio)	Selective cultivation	Transmission risk	- Sampling: DWTPs Analyses: SINTEF, commercial labs - Data: Norwegian DWTP Registry	Low (very low prevalence)
	Tap water	**WHO/ UNICEF JMP MICS6** (Table AIII.1)	Enumeration of *E. coli*	Cultivation	Transmission risk	- Sampling: Community Officer - Analyses: Commercial water labs - Data: Norwegian DWTP Registry	Low (very low prevalence)
Human waste	WWTPs: Untreated wastewater	**WHO One Health survey** (Appendix AII.2)	ESBL-*E. coli* (concentration, ratio)	Selective cultivation	Proxy AMR carriage	- Sampling: WWTPs - Analyses: Commercial water labs - Data: NIVA	High (international contribution)
		WHO polio surveillance	Poliovirus	PCR	Proxy AMR carriage	- Sampling: WWTPs - Analyses: Commercial water labs - Data: NIVA	Low (very low prevalence)
		US CDC NWWS (Table AII.5)	Various (SARS-CoV-2, ARGs)	qPCR; meta-genomics	Proxy AMR carriage	- Sampling: WWTPs - Analyses: Commercial water labs - Data: NIVA	Intermediate
	WWTPs: Treated wastewater	**EU Umbrella project**** (Table AII.5)	SARS-CoV-2, ARGs	qPCR, metagenomics	Transmission risk	- Sampling: WWTPs - Analyses: Commercial water labs - Data: NIVA	High (will probably be a mandatory EU WWT Directive)
			Antimicrobial residues	Liquid chromatography	Risk of AMR development		
	WWTPs: Activated sludge	**Codex Alimentarius** (Appendix AII.3) and **WHO/FAO Expert Meeting** (Appendix AII.4)	Salmonella, Campylobacter, E. coli, Enterococci	Cultivation; molecular methods	Transmission risk	- Sampling: WWTPs - Analyses: NMBU - Data: NIVA	Intermediate

Main matrix	Sample site	International setting	Microbiology target	Method	Goal	Proposed responsible institution in Norway	Suggested priority
	Hospital wastewater outlets	Single studies	Various	Various	Proxy AMR prevalence	- Sampling: Hospital sanitation technicians - Analyses: Commercial water labs - Data: NIVA	Low
Animal waste	Untreated wastewater from slaughterhouses	**WHO One Health survey** (Appendix AII.2)	ESBL-*E. coli*	Selective cultivation	Proxy for AMR prevalence	- Sampling: Community Officers - Analyses: Commercial water labs - Data: Norwegian Food Safety Authority	Intermediate
Irrigation water	For leafy greens, berries, etc.	**Codex Alimentarius** (Table AII.3) and **WHO/FAO Expert Meeting 2018** (Table AII.4)	*Salmonella*, *Campylobacter*, *E. coli*, Enterococci	Selective cultivation; molecular methods	Transmission risk	- Sampling: NIBIO - Analyses: Commercial water labs - Data: Norwegian Food Safety Authority	High
Manure	At terrestrial animal farm	**Codex Alimentarius** (Table AII.3) and **WHO/FAO Expert Meeting 2018** (Table AII.4)	*Salmonella*, *Campylobacter*, *E. coli*, Enterococci	Selective cultivation; molecular methods	Transmission risk	- Sampling: Animal farmer - Lab analyses: NMBU - Data: Norwegian Food Safety Authority	Intermediate
Aquaculture	Shellfish	**EU Regulation (**EC 2019/624	*E. coli*	Most probable number (ISO 16643-3:2005)	Transmission risk	- Sampling: Aquaculture farmers - Analyses: Norwegian Institute of Marine Research - Data: Norwegian Food Safety Authority	High
	Water adjacent to shellfish production	EU Directive 2006/113	No microbiology target	-			

Main matrix	Sample site	International setting	Microbiology target	Method	Goal	Proposed responsible institution in Norway	Suggested priority
	Sediments under fish farms	Single studies	Antimicrobial residues	Flow cytometry	Risk for AMR development	- Sampling: Aquaculture farmer - Lab analyses: Norwegian Institute of Marine Research - Data: Norwegian Food Safety Authority	High
		Codex Alimentarius (Table AII.3), **WHO/FAO Expert meeting 2018** (Table AII.4)	Various*		Transmission risk		
Soil	Different types of soil	**EU LUCAS soil** (Table AII.1)	- Biodiversity (bacteria and archaea) - Pollutants (pesticides)	16S rDNA; Pesticide residues	Risk for AMR development	- Sampling: JOVA programme - Lab analyses: NMBU - Data: NIBIO	Intermediate
River water	Before and after the capital city	**WHO One Health Survey** (Table AII.2)	ESBL- *E. coli*	Selective cultivation	Transmission risk	- Sampling: Norwegian River Water Programme (additional sites) - Lab analyses: NIVA - Data: NIVA	High (international contribution)
		GEMS/Water (Table AIII.2)	*E. coli*, total coliform count (level 2 monitoring)	Cultivation	Transmission risk	- Sampling: Norwegian River Water Programme (additional sites) - Lab analyses: NIVA - Data: NIVA	Intermediate (probably low prevalence)
Lake water	Various	**GEMS/Water** (Table AIII.2)	Various	Cultivation	Transmission risk	- Sampling: NIVA and NINA - Lab analyses - Data: NIVA	Intermediate (probably very low prevalence)
Recreational water	Bathing water (beach, river, lake), public swimming pools, etc.	**EU bathing water directive**	Colony forming units (CFU)/100ml coliforms, enterococci	Cultivation	Transmission risk	- Sampling: Local community - Lab Analyses: Commercial water labs - Data: Norwegian Environment Agency and Norwegian Water Resources and Energy Directorate	High (probably intermediate prevalence + risk transmission)

Main matrix	Sample site	International setting	Microbiology target	Method	Goal	Proposed responsible institution in Norway	Suggested priority
						(NVE) https://vann-nett.no/portal/	

*Codex Alimentarius Guideline on AMR surveillance in food production states 'Selection of the target microorganisms and resistance determinants should be considered based on their relevance to food safety and public health. Bacterial species may include foodborne pathogens (*Salmonella, Campylobacter*), and indicator bacteria such as *E. coli* and enterococci.

GLASS = Global Antimicrobial Surveillance System, JMP MICS6 = Joint Monitoring Programme
NWWS = National Wastewater Sewage Surveillance
NIVA = Norwegian Institute for Water Research
NIBIO = Norwegian Institute of Bioeconomic Research
NMBU = Norwegian University of Life Sciences
NINA = Norwegian Institute for Nature Research
LUCAS = Eurostat Land Cover/Use Statistics
ESBL = Extended spectrum beta-lactamase
NWWS = National Wastewater Sewage Surveillance

13. Uncertainties

The scientific knowledge of the natural characteristics of AMR in the environment, and how such characteristics have been and are influenced by human activities and pharmaceutically produced antimicrobials, is currently far less developed than the knowledge base of AMR in clinical settings. The studies of environmental resistance are often small-sized, of limited duration, and most often report on point prevalence. The choices of study sites appear somewhat arbitrary and fragmented. There is limited coordination at the international level of studies, study design, standards and reporting formats. The complexity of the environmental dimension of resistance and the current state of the art in the field of environmental AMR results in several uncertainties that affect our ability to understand and evaluate the opportunities for surveillance, to prioritize among efforts, to propose the most resource-efficient methods, and to reduce uncertainty. The various forms of uncertainties also imply that assumptions and judgements will have to be made.

Uncertainties that affect the understanding of the environmental dimensions of resistance include:

- Natural diversity and complexity of the ecosystems
- Different levels of anthropogenic exposure and exposure routes
- Multiple pathways and historical contingencies that shape observed resistance patterns
- Effects of chance events
- Various interfaces in space and time that facilitate resistance development/transfer
- Combined effect of various sources of pollutants in resistance selection
- Host effects of sub-lethal exposures to antimicrobials over time
- Multiple global transmission pathways of resistance traits
- Resistance dynamics in environments not exposed to antimicrobials
- Effects of resistance traits on host fitness and evolutionary trade-offs and counterselection
- Dynamics of resistance in space and over time

Uncertainty also arises due to limited development and availability of standardized methods.

- Lack of robust and uniform methods for identification/description of resistance development in non-clinical microbial cells, species, and populations.
- Limited ability to backtrack the emergence, amplification pathways, persistence, and decay routes for resistance traits (limited opportunity to backtrack excludes opportunity to establish causality)
- Variable approaches to handle natural and technical variation
- Variable data storage and reporting formats of studies
- Variable study design for similar environmental samples

- Unclear approaches to how clinical, veterinary clinical and environmental datasets could meaningfully be combined to understand resistance developments across environments

Uncertainty related to the use of collected data

- The materialized benefits from integrated surveillance for antibiotic resistance control
- The extent of risk reduction achievable
- The effective reduction of antibiotic resistance in the environment as a consequence of the implementation of control measures

Given the high complexity of environmental ecosystems, any surveillance effort will need to make strong priorities and exclusions. This creates model uncertainty. See also (Bengtsson-Palme et al., 2021) for a further discussion on uncertainty.

14. Conclusions, answer to the questions in the mandate

Based on the above information gathering and assessment, the VKM concludes as follows to the questions raised in the mandate.

> Summarize the knowledge base supporting the definition and description of environmental resistance and propose a definition of acquired resistance in environmental bacteria based on established clinical definitions.

The knowledge base supporting the definition and description of environmental resistance is summarized in chapters 6–10. For single microbial isolates of environmental origin, the definition, taxonomy, and clinically oriented methods developed for describing the level of antimicrobial susceptibility (MIC, MBC, MSC) can be directly applied. For those environmental isolates with a known history of causing human disease (e.g. zoonoses, waterborne pathogens, environmental pathogens), resistance acquisition and dynamics can be expressed through clinical breakpoints and ECOFF values.

For observing and communicating changing patterns of resistance in samples from complex microbial communities, a new set of descriptors should be developed, standardized, and harmonized at the global level. Such descriptors should be supported by clear taxonomy and standardized methods and reporting formats. The descriptors will focus on the resistome present in the microbial community of the sampled environment rather than the resistance traits present in single cells extracted from that environment.

> Evaluate available methods for resistance determination and the extent to which these are suitable for environmental samples, including the pros and cons of culture-based methods versus metagenome/whole genome-based methods (phenotype versus genotype).

The methods used for resistance determination and their pros and cons are summarized in chapter 8 and further discussed in chapter 12. Numerous methods are available, and their suitability will depend on the aim of the analyses. There is no single optimal method for determining AMR in environmental samples. Culture-based methods are the gold standard for clinical isolates and are preferred also for environmental isolates with a known history and pathogenic potential. Culture-based methods can also be combined with various DNA/RNA/protein-based methods.
Several approaches are available in situations when single isolates cannot be obtained or the bacterial diversity in the sample exceeds the processing capacity. For such microbial community-focused analyses, the main approach is metagenomic and is based on sequencing of extracted DNA. Current limitations in many metagenomics-based studies are the limited opportunity to extrapolate resistance phenotypes from genomic/genetic data and the dependency on curated databases for the identification of known resistance traits. A clear advantage is the unbiased/untargeted approach, in contrast to most culture-based

approaches that will target a defined subset of the microbes present in a given environmental sample. Culture-based methods are also quickly saturated by the number of samples that can be processed and will overlook the majority of AMR determinants in an environmental sample. Ultimately, the suitability of a method will depend on the research question being asked and will entail a trade-off between the resources required and the amount and type of data produced and processed.

> Evaluate possibilities and limitations in sampling design and methods for sampling, sample material, and sample selection, including how this is solved in today's NORM/NORM-VET approach.

The experimental design and methods for sampling will be defined by the research question and adapted to available resources. As discussed in chapter 12, resource-effective design and prioritization is an integral part of surveillance. Hence, new surveillance efforts must combine current sampling practices with the most pressing knowledge gaps and risk scenarios as well as with ongoing developments in the research communities addressing standards, variability, uniformity, and representativeness. In all cases, experimental limitations should be addressed and clearly communicated. It is expected that sampling will be coordinated with other control systems and programmes, for example, internal control sampling in WWTPs and drinking water plants as well as the surveillance of freshwater sources and beaches. Coordinated collection of samples will reduce the costs for a NORM-ECO programme. Such coordination will also enable more insights into the sampled material and will place the AMR data in a wider context.

The sampling design and choice of methods in NORM/NORM-VET have been refined over the years, considering method development, new technologies, revised international standards, and recommendations (see chapter 6). Results are communicated through reports on a yearly basis. Some important groups of culturable bacteria are analyzed yearly (for example *E. coli* from human blood cultures), while other groups vary between years.

> Develop an overview of existing and planned approaches to environmental resistance monitoring nationally and internationally and assess how these can best be used in a potential NORM-ECO approach.

An overview is presented in chapter 6 and discussed in chapter 12. Currently, there are four main international perspectives that will impact and guide Norway’s choice for how to survey AMR in the environment. The first is the WHO One Health Survey that is part of the Global AMR Surveillance System (GLASS) using ESBL *E. coli* as comparator across compartments and river water and wastewater being the environment sampled. The second is the recent Codex Alimentarius guideline on surveillance of AMR in food production environments mentioning irrigation water, manure, and sediments under fish farm. The third are EU initiatives on surveillance of AMR and antibiotics and their residues within the environment as part a response to the Strategy on Pharmaceuticals in the Environment, the revised EU AMR action plan, and the Green Deal initiative. Neither the WHO One Health Survey nor the provisions in the Codex guidelines are mandatory but still represent political direction. Also, and depending on the nature of regulations, what the EU decides or advises Norway either

has to follow or is strongly encouraged to do. Lastly, the recent UN Environment report (2022) (https://www.unep.org/resources/report/summary-policymakers-environmental-dimensions-antimicrobial-resistance expresses the immediate need for concerted international environmental action to i) enhance environmental governance, planning and regulatory frameworks, ii) identify and target prioritized AMR-relevant pollutants, iii) improve reporting, surveillance, and monitoring, and iv) prioritize financing, innovation, and capacity development. This latter summary report specifies directions and contextualizes a NORM-ECO programme but do not detail a specific approach.

> Base work on a comprehensive assessment of the knowledge base, identifying the main challenges, opportunities, and added value of establishing a NORM-ECO monitoring programme.

Chapter 3 and 12 discuss the premises behind and the broader opportunities and limitations of environmental surveillance. The premises that must be met for the successful establishment of surveillance include that the rationale for surveillance is clearly defined, that the specific knowledge gaps addressed are clearly stated, that the surveillance effort is transparent in its description of uncertainty and risk, that the surveillance effort is based on resource-effective prioritization, that the most risk-relevant targets/sites/conditions have been identified in processes involving all relevant stakeholders, that the surveillance effort is an integrated part of a One health framework, and that data are collected through standardized methods and reporting that also provide its context and variability.

The main methodological challenges and opportunities are presented in chapter 8. At the general level, the main challenges include the complexity of the biological systems considered – which prevent causality from being established – the differences among stakeholders in the rationale for surveillance leading to different needs/cost/benefit analyses, the current plurality of research design and agendas, initiatives, and programmes, and the lack of a single coordinated effort at the international level.

The opportunities and added value of monitoring include establishing systematic and coordinated efforts at the international level that will build knowledge across environments that closes key knowledge gaps and reduces uncertainty, supporting and contributing to international initiatives on One Health and environmental resistance, including contributing at an early phase to developing standards, strengthening the basis for the National Strategy against AMR, establishing a One Health orientation towards AMR in Norway, and developing a resource-effective approach by drawing on existing surveillance/sampling efforts. This will contribute to an internationally-harmonized, step-wise, and scalable approach to a global health problem.

> After clarifying added value, suggest how further work in VKM can develop the programme, including identification of manageable selection of environment, samples, species, and methods that can provide a basis for standardization and time series.

Further work in VKM can inform and support the development of a programme through:

a. Developing the rationale and corresponding scientific framework of a programme based on the key premises, limitations, and opportunities presented in this report.
b. Facilitating stakeholder processes that identify and articulate the key knowledge gaps and risk scenarios relevant to Norway.
c. Considering how current international, EU, Nordic, and national research programmes could contribute to defining relevant analytes in a Norwegian to international context.
d. Drawing on available scientific networks that focus on environmental resistance (e.g. NORSE).
e. Engaging with NORM and NORM-VET programmes to examine if, and if so how, a broader set of environmental isolates could be included.
f. Identifying other environmentally oriented surveillance programmes in Norway for which existing sampling could be made multi-purpose.
g. Aligning the programme with international initiatives regarding both methodology, standardization, and reporting formats.
h. Suggesting how the programme will be organized by a responsible agency and institution.
i. Developing a digital platform for sharing of resistance data (findable, accessible, and formatted for comparative usage).
j. Undertaking uncertainty analyses to support prioritization and communication of risk.

As a first practical step, the rationale, risk scenarios, and knowledge gaps should be established. This provides the basis for the manageable selection of environments, samples, species, and methods. The practical aspects should be developed in parallel and in collaboration with ongoing international initiatives.

An early focus on sampling efforts is expected for aquatic systems such as runoff, sewage, rivers, and drinking water. Some of these would focus on sources of resistance that enter the environment, rather than representing the resistance situation as such. Any activity should therefore be explicit about what part/process of the One Health dimension being addressed. Water is also a vehicle for the transport of microbes (including antimicrobial resistant microbes) from one location to another. Other interfaces of particular interest may be farmland and feeding places for grazing domestic and wild animals, including feeding or hatching places for migrating birds). Sampling of other environmental matrices including those affected by wildlife should be decided upon on a cyclical basis.

15. Further considerations

Based on the assessment made in this report, and further addressing question 6 in the mandate, the following considerations are offered:

There is a scientific rationale for increased surveillance efforts that can:

- Strengthen the One Health orientation on a pressing global issue and increase our knowledge of the effects of anthropogenic practices on the environment
- Contribute to the current international initiatives that include the environmental pillar of One Health
- Contribute to the further development of relevant programmes at the EU level that include environmental resistance

From a scientific perspective, there is a clear opportunity to further develop the knowledge base of environmental resistance in Norway through prioritizing among research questions, designs and key analytes ensuring that the knowledge produced:

- contributes with longitudinal data collected in a systematic manner
- is resource effective and builds on other environmental and clinical sampling programmes
- is developed in a step-wise, adaptable, and scalable manner
- benefits from rapid advances in technology and data processing capacity
- ensure representative sampling
- address the scientific questions as further as outlined in the answer to mandate 6

The scientific community may also consider how to strengthen the bridges the human, animal, and environmental disciplines of research to develop a shared understanding of the most pressing knowledge gaps, the most AMR-relevant pollutants, and the most risk-relevant scenarios in Norway.

15. Data gaps

Data gaps represent limitations in current knowledge that can be reduced through further research and analyses.

Biological aspects

- The overall diversity (type, mechanism, concentration) of resistance-driving agents in the environment (e.g. cleaning chemicals, various drugs including antimicrobial agents, etc.) is only described to a limited extent. More knowledge is needed regarding their role in positive selection of and co-selection of antimicrobial resistance traits.
- Combination toxicology/resistance (mixture toxicology)-based studies in the environment are not yet fully developed but are of importance given the expected simultaneous exposure to different resistance drivers in human-influenced environments. These include metals, solvents, oils, PHAs, pesticides, microplastics, biocides, and antimicrobials (Reygaert, 2018).
- Exposure rates are only described to a limited extent in the environment. The rate of decomposition and dynamics of resistance (bacteria, genes, and selective agents) dispersed from anthropogenic environments are not widely known, including the long-term effects of continuous discharges (e.g. through sewage).
- There is limited understanding of where in the environment and under what circumstances the critical steps occur that lead to the emergence of new forms of resistance in pathogenic bacteria.
- There is bias in the data on environmental resistance towards bacterial species that are culturable and that have clinical counterparts.
- There is limited availability of studies that follow environments over time and that include spatial distributions.

Methodological aspects

- There is a lack of an internationally standardized methodological framework for sampling, handling, and presenting data in non-clinical environments limits opportunities for comparative analyses.
- There is a lack of robust methods for distinguishing between acquired resistance, mobile resistance, and inherent resistance in novel environmental cells or isolates with limited history. Also, the methods for phenotypic resistance determination in specimens that cannot be cultured in the laboratory are not fully developed.
- Methodological challenges limit the focus to culturable anaerobes
- Several DNA sequence databases exist for resistance traits affecting a given comparative DNA analyses. The content of these databases is not identical, reflecting differences in inclusion criteria and curation.
- The use of emerging experimental methods/technologies in combination with artificial intelligence and machine learning is yet to be fully explored. New technologies and

new combinations of technologies including data management and analysis now quickly change the opportunities for cost-effective monitoring of resistance traits.
- Multipurpose sampling has been explored only to a limited extent.
- Limited international coordination has led to a research practice of point prevalence-based studies characterized by heterogeneity in methods and samples, targets, and bacterial species.
- There are severe limitations to sampling due to scale (e.g. global) versus funding for time-limited local studies.
- There are severe limitations due to the saturation of sampling efforts. The high density of bacteria in solid samples such as soil/sediment and gut content (10^8–10^9 cells/gram) quickly surpass the cultivation efforts or the amount of DNA that can be analyzed when larger geographical areas are of interest.

Operational/reporting aspects

- Standardized storage and reporting formats are necessary for digital processing and comparative analysis of larger datasets.
- The terminology describing environmental resistance may not yet be fully available.
- There is limited availability of systematic reviews and knowledge summaries – leading to poor contextualization of many studies.
- There is limited discourse on the most pressing knowledge gaps and risk scenarios, and how stakeholders recognize, engage, and contribute to the issue.
- There is limited coordination of research efforts beyond the national level.
- There is uneven distribution of capacity between countries and researchers.

APPENDIX I – Systematic review

Method used for the systematic review of the literature addressing mandate questions 1-6.

During 2021, a structured search in the published literature was conducted across four main databases – Web of Science, Medline, Scopus, and Embase – using the terms in the PICO scheme (See below; Table 1). After consultation between authors (SY and KMN) and library staff, the search was initially limited to reviews, and later to systematic reviews only, because the number of original articles was >100,000 and most of them were irrelevant for the purpose of this report. After identifying all systematic reviews meeting the inclusion criteria, titles and abstracts were initially screened by author SY in cooperation with ALW to identify relevant studies for further review. Articles were then sorted and screened based on defined inclusion and exclusion criteria (see below).

Protocol

We did not prepare and publish a protocol for the systematic review, but had defined inclusion and exclusion criteria.

Inclusion and exclusion criteria

- The search was limited to include only environmental articles regarding AMR
- Only articles written in English
- Published during the time period 2011–2021.
- Systematic review articles (but were screened for any citation relevant to the review)

Evaluation of risk bias in systematic overview (Risk Of Bias)

For evaluation of the risk for bias (ROB), we used the JBI Critical Appraisal Checklist for Systematic Review and Research Syntheses (JBI, Faculty of Health and Medical Sciences, University of Adelaide, South Australia) https://jbi.global/critical-appraisal-tools

The following questions were answered by Yes, No, Unclear, or Not applicable:

Is the review question clearly and explicitly stated?

Were the inclusion criteria appropriate for the review question?

Was the search strategy appropriate?

Were the sources and resources used to search for studies adequate?

Were the criteria for appraising studies appropriate?

Was critical appraisal conducted by two or more reviewers independently?

Were there methods to minimize errors in data extraction?

Were the methods used to combine studies appropriate?

Was the likelihood of publication bias assessed?

Were recommendations for policy and/or practice supported by the reported data?

Were the specific directives for new research appropriate?

Based on these evaluations we initially included five articles, excluded four articles, and placed one article under the category "seek further info". After discussion between authors ALW and SY, we agreed to include that article (Coertze and Bezuidenhout, 2019).

Data extraction in the systematic review

Titles and abstracts were screened based on the inclusion-exclusion criteria by two independent reviewers (Authors SY and ALW). For any disagreements between the two reviewers, a third reviewer was used as a tiebreaker.

Our systematic review data extraction was based on the following six included articles:

- Andrade L, Kelly M, Hynds P, Weatherill J, Majury A, O'Dwyer J. Groundwater resources as a global reservoir for antimicrobial-resistant bacteria. Water Res 2020;170:115360
- Bordier M, Uea-Anuwong T, Binot A, Hendrikx P, Goutard FL. Characteristics of One Health surveillance systems: A systematic literature review. Prev Vet Med 2020;181:104560
- Chau KK, Barker L, Sims N, Kasprzyk-Hordern B, Budgell EP, Harriss E, Crook DW, Read DS, Walker AS, Stoesser N. Wastewater surveillance of antimicrobial resistance in human populations: a systematic review. Preprint, not peer-reviewed. Doi:10.20944/preprints202010.0267.v1
- Coertze RD, Bezuidenhout CC. Global Distribution and current research of AmpC beta-lactamase genes in aquatic environments: A systematic review. Envir Poll 2019;252: 1633-1642
- Hamilton KA, Garner E, Joshi S, Ahmed W, Ashbolt N, Medema G, Pruden A. Antimicrobial-resistant microorganisms and their genetic determinants in stormwater: A systematic review. Env Sci Health 2020; 16:101-112
- Hassoun-Kheir N, Stabholz Y, Kreft JU, de la Cruz R, Romalde JL, Nesme J, Sørensen SJ, Smets BF, Graham D, Paul M. Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. Sci Tot Env 2020;743: 140804

Data synthesis

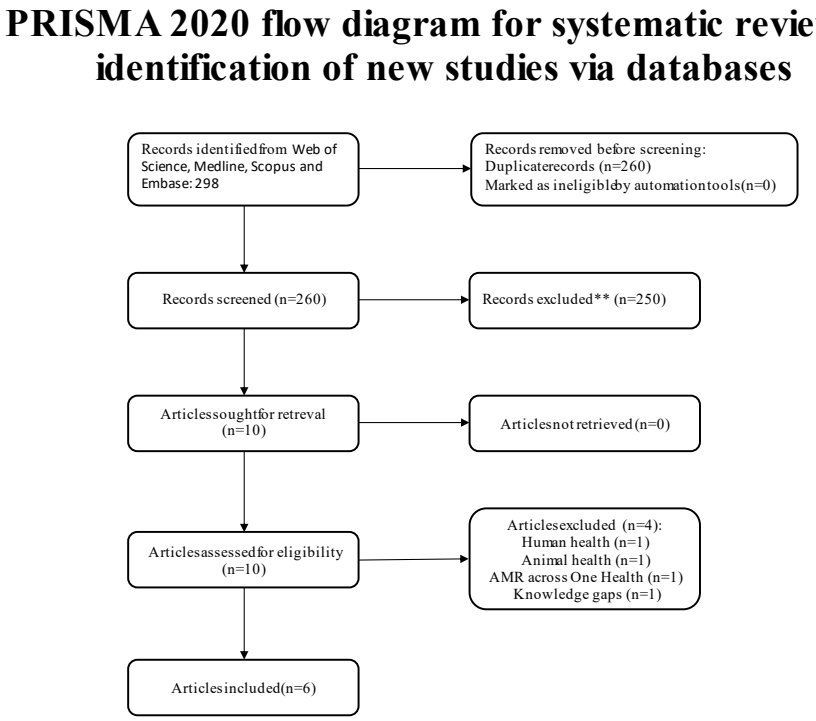
For data synthesis we used the six included articles.

The following data points were extracted in a structured way (Excel sheet):

Article information - e.g., database, citation, title, abstract
Author information - e.g., Author names, address/country
Environmental setting: water/wastewater/sludge/wildlife
Study Methods - e.g., design, study period
Study Population - e.g., country, sample size
AMR related – e.g. AMR testing methods, susceptibility
ARG selection

Results of the systematic literature search

Figure A1. Flow diagram for new systematic reviews that included searches of databases and registers only



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n7

Table 1. PICO scheme for the search strategy in this report.

What is the question that the SR addresses	Question in PICO format				Known relevant studies
	Population (pasient)	**Intervensjon (tiltak)**	**Comparison (sammenligning)**	**Outcome (utfall)**	
Which methods can be used in a surveillance system of AMR in non-clinical environments Hvilke metoder kan brukes i et overvåkningssystem av antimikrobiell resistens i ikke-kliniske miljøer?	AMR Anti-microbial resistance	Environment Wildlife	**Time/temporal dynamic** Spatial epidemiology Sampling Monitoring Mixture Decay Standardization		Yamamoto T, Hayama Y, Hidano A, Kobayashi S, Muroga N, Ishikawa K, Ogura A, Tsutsui T. Sampling strategies in antimicrobial resistance monitoring: evaluating how precision and sensitivity vary with the number of animals sampled per farm. PLoS One. 2014 Jan 23;9(1):e87147. doi: 10.1371/journal.pone.0087147. Silley P, Simjee S, Schwarz S. Surveillance and monitoring of antimicrobial resistance and antibiotic consumption in humans and animals. Rev Sci Tech. 2012 Apr;31(1):105-20. doi: 10.20506/rst.31.1.2100. Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016). Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. Frontiers in microbiology, 7, 1728. https://doi.org/10.3389/fmicb.2016.01728

APPENDIX II – Environmental AMR surveillance initiatives

Table AII.1. EU Land Use Cover Area (LUCAS)* soil module

Link to protocol	**European Commission, Joint Research Centre, Fernandes-Ugalde, O., Scarpa, S., Jones, A., et al., *LUCAS soil 2022: ISSG planning document*, Publications Office, 2021, https://data.europa.eu/doi/10.2760/74624**

Main matrix	Sample site	Survey-specific site selection	Targets	Method**	Frequency	Goal	Data repository	Suggested Norwegian setting	Suggested Norwegian repository
Soil	Cropland Grassland Woodland Wetland Shrubland Bare land	Topsoil	- Core analyses[1] Bulk density[2] - Biodiversity, including **ARGs**[3] - Pollution including **antibiotics**[4]	ARGs (metagenomics)[5] Antibiotics (LC/MC/MC)	Depending on expected change/year	Risk for AMR transmission between compartments Risk for AMR development within the environment	European Soil Data Centre (ESDAC) at the European Joint Research Centre	Norwegian Agricultural Environmental Monitoring Programme (JOVA) + Recommended more extensive surveillance partly aligned to LUCAS soil[6]	Norsk institutt for bioøkonomi (NIBIO)

*Eurostat Land Cover/Use Survey statistics (LUCAS) methodology: https://ec.europa.eu/eurostat/web/lucas/methodology

[1]Core analyses LUCAS soil: Organic carbon, total nitrogen, P, K, S and pH (Ca, Na, Mg, Mg, H). Electrical conductivity in saline-prone areas (i.e. irrigated land, costal agricultural plots). Particle size and coarse fragments.

[2]Bulk density: in soils with compaction problem (> 1.3 g/cm^3)

[3]Biodiversity and genetic assessments: DNA analyses for biodiversity index. Key genes for functional assessment. Presence of antibiotic-resistance genes.

[4]Soil pollution module (budgetary restrictions allowing): metals, antibiotics, plastics, industrial chemicals

[5] (Orgiazzi et al., 2017)

[6]Nibio rapport 2021;7(14); Jordsmonnet vi lever av – Forslag til system for dokumentasjon og rapportering av jordsmonnets tilstand og endring

Table AII.2. WHO One Health Survey (Tricycle project), WP3: ESBL-*E. coli* in the environment									
Link to protocol		https://www.who.int/initiatives/glass/glass-modules-7							
Matrix	**Sample site**	**Survey specific site selection**	**Target**	**Method**	**Frequency**	**Goal**	**Data repository**	**Suggested Norwegian setting**	**Suggested Norwegian repository**
Drinking water (optional)	Drinking water treatment plant	Serving capital + sentinel city[1]	ESBL-E. coli[2]	Selective cultivation[3]	8-12 samples /month	Risk for transmission between compartments	GLASS One Health IT platform	Sampling under Norwegian Law (Drikkevannsforskriften)	Vannverks-registeret NIPH)
Untreated wastewater	Wastewater treatment plant	Serving capital + sentinel city[1]	Antibiotic residues (optional)	Subset frozen[4]		Proxy for antimicrobial use			
			ESBL-E. coli[2]	Selective cultivation[3]	8-12 samples /month	Proxy for AMR carriage	GLASS One Health IT platform	Sampling under Norwegian Law (Forurensningsloven)	NIVA
Drainage	Wastewater from slaughterhouse	Within capital + sentinel city[1]	ESBL-E. coli[2]	Selective cultivation[3]	8-12 samples /month	Proxy for AMR carriage	GLASS One Health IT platform	Sampling under Norwegian Law (Forurensningsloven)	NIVA
River water		Upstream, downstream of capital + sentinel city[1]	ESBL-E. coli[2]	Selective cultivation[3]	8-12 samples /month	Risk for transmission between compartments	GLASS One Health IT platform	Norwegian River Water programme (Norwegian Environment Agency)	NIVA

[1]A sentinel city is one with approximately 100,000 residents

[2]Number of colonies on plates with and without antibiotic addition, translated into concentration and proportion of ESBL-E. coli vs. non-ESBL-E. coli

[3]24 h composite sample or 3x grab samples. Membrane filtration. Tryptone Bile X-glucoronide agar (TBX) with/without cefotaxime at 4 µg/mL. Phenotypic ESBL confirmation. Antimicrobial susceptibility testing for selected antibiotics, PCR (for most prevalent ESBL-genes), and whole genome sequencing of all or a randomly selected subset of isolates (optional).

[4]Subset of samples immediately frozen and shipped to a specialized laboratory for performing liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) or similar.

Table AII.3 Codex Alimentarius Guidelines on integrated monitoring and surveillance of foodborne AMR, which includes food production environments*

Link to guideline		CXG 94-2021							
Matrix	**Sample sites mentioned**	**Survey specific site selection**	**Target[1]**	**Method**	**Frequency**	**Goal**	**Data repository**	**Suggested Norwegian setting**	**Suggested Norwegian repository**
Food production environments*	Soil, water, litter and animal bedding, organic fertilizer, sewage, or manure[2]	**Not specified**	Foodborne pathogens (*Salmonella*, *Campylobacter* or other) Indicator bacteria (*E. coli* and enterococci)	Cultivation, phenotypic antimicrobial susceptibility testing Molecular methods	To be specified at the national level	Risk for transmission between compartments	National level	NORM/NORM-vet/NORM-ECO	Norwegian Food Safety Authority

*Food production environment as defined by the Codex Guideline (CXG 94-2021): *"The immediate vicinity of the food chain where there is relevant evidence that it could contribute to foodborne AMR",* and exemplified only in footnote 6 (Page 8)[2]

[1] "*Target microorganisms from aquatic animals and food of non-animal origin may be determined based on available scientific evidence and/or relevance to public health."* (Page 8, CXG 94-2021))

Table AII.4 WHO/FAO Expert Meeting in collaboration with OIE on Foodborne AMR – Role of the Environment*, Crops and Biocides									
Link to Expert Meeting Report		https://apps.who.int/iris/handle/10665/332387							
Main matrix	**Sample sites mentioned**	**Survey specific site selection**	**Target**	**Method**	**Frequency**	**Goal**	**Data repository**	**Suggested Norwegian setting**	**Suggested Norwegian repository**
Food production environment	Soil	In production of crops intended to be eaten (by humans or animals) raw or with minimal processing	ARGs	Microbiome/metagenome, qPCR	Not specified	Risk for transmission between compartments	Not specified	Norwegian Food Safety Authority	NMBU
	Irrigation water		Antimicrobial residues	*"Chemical analyses"*		Risk for AMR development within the environment			NIVA
	Manure								NMBU
	Sewage								NIVA
	Sewage sludge								NIVA

	Aquaculture	Aquaculture water Sediments	Not specified	Not specified		Risk for AMR development within the environment			Norwegian Institute of Marine Research
*Adjusted to statements on surveillance or in line with level of emphasis on specified food production environments in the expert report									

Table AII.5 Research initiatives on AMR surveillance in the environment

Initiative	Main matrix	Survey specific site selection	Target	Method	Frequency	Goal	Data repository	Suggested Norwegian setting	Suggested Norwegian repository
Global sewage study[1]	Untreated wastewater	Urban	ARGs	1L within 24 h Metagenomics	2 consecutive days/year	Proxy for AMR carriage	Danish Technical University	Commercial water laboratories	NIVA
EU Umbrella study: Feasibility Assessment[2]	Untreated wastewater	not specified	SARS-CoV-2, AMR[1]	24 h composite Realtime PCR	A day/round	Proxy for AMR carriage	EU Joint Research Center	Commercial water laboratories	NIVA
US CDC NWSS[3]	Untreated wastewater, primary sludge	Communal Sewage sludge treatment plants (SSTPs, institutions, low-resource waste systems	SARS-CoV-2, AMR (2 CDC projects, and mentioned from CDC representative to TATFAR[3])	qPCR, digital droplet PCR, culture-based	Not specified	Proxy for AMR carriage	US CDC	Commercial water laboratories	NIVA
CORNELIA project (Norway)	Various	To be defined	To be defined	Metagenomics, cultivation	To be defined	Various		NMBU	NMBU

[1]Mentioned from DG Environment representative during Transatlantic Taskforce for AMR as of October 2020 (Table 9, Page 40 in Joint Research Centre Technical Report: SARS-Cov-2 Surveillance employing Sewage Towards a Sentinel System 2021)(Gawlik BM, 2021)

[2] (Hendriksen et al., 2019)

[3] Transatlantic Taskforce on Antimicrobial Resistance 2016-2021 Progress Report. US CDC 2021.

APPENDIX III – Established surveillance into which AMR could be added

The UN system runs three highly relevant environmental surveillance systems that could potentially also include the surveillance of AMR.

Since the mid-1990s, more than 100 countries have carried out **UNICEF Multiple Indicator Cluster Surveys (MICS) on water quality** (Table AIII.x). This programme, together with the WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene, has started the MICS6 module on testing household drinking water for levels of *E. coli*. (https://mics.unicef.org/methodological_work/3/WATER-QUALITY)

A feasibility study on testing *E. coli* for ESBL production in MICS6 has been performed in Bangladesh, funded by Norway (ALW pers. com. with responsible person in the WHO).

In Norway, routine surveillance of drinking water quality is performed by community drinking water plants in line with requirements defined in the Norwegian regulation on drinking water (https://lovdata.no/dokument/SF/forskrift/2016-12-22-1868?q=drikkevann) and funded by consumers.

The **UNEP Global Environment Monitoring System for freshwater (GEMS Water)** (Table AIII.xx) has surveyed water quality around the world for decades (https://www.unep.org/explore-topics/water/what-we-do/monitoring-water-quality). Well-established variables are indicators of nutrients (phosphorous, nitrogen), oxygen levels, pH, toxicants (heavy metals, pesticides, organic pollutants), and electrical conductivity. Adding biological indicators is advised, such as measuring levels of certain fish species, invertebrates, algae, and microbial pollutants (cyanobacteria, *E. coli*, and total coliform count) (*UN Environment 2017. A Framework for Freshwater Ecosystem Management. Volume 2: Technical guide for classification and target-setting*). To our knowledge, there is no initiative to include AMR in GEMS Water. However, there might currently be a political space within UNEP and beyond to work on AMR related to ambient water quality, as exemplified by the recent UNEP report on environmental impacts of AMR https://wedocs.unep.org/bitstream/handle/20.500.11822/34797/K2003026.pdf?sequence=1&isAllowed=y), and this may lead to discussions on how to include AMR surveillance into different streams of UNEP work.

GEMS Water data is handled and presented by GEMS Stat (https://gemstat.org/about/).

Similar to the GEMS/Water system, The Norwegian Institute for Water Research (NIVA) is contracted from the Norwegian Environment Agency to perform surveillance of water quality of rivers, lakes, and the sea, with a focus on toxic substances and ecosystem health. https://www.niva.no/tjenester/overvakningsprogrammer. Apparently, *E. coli* is not included in routine surveillance of ambient water quality in Norway (Moe TF, Persson J, Bækkelie KAE et al. Overvåkning av referanseelver 2018. Basisovervåking I henhold til Vannforskriften. 2019. http://hdl.handle.net/11250/2601030), (Kaste Ø. Skarbøvik E, Greipsland I et al. Elveovervåkningsprogrammet - vannkvalitetsstatus og – trender 2017. http://hdl.handle.net/11250/2588692)

The **WHO Polio Programme** (Table AIII.3) traditionally uses acute flaccid paralysis surveillance for rapid detection of the polio virus. Stool samples of patients in question are tested at laboratories especially designated for polio detection. Approximately 1/200 carrying the virus are symptomatic, hence the virus can silently circulate without being picked up by the paralysis surveillance. Therefore, **environmental polio surveillance** testing sewage and open drains from populations where polio is likely to be present has been initiated, with a special focus on areas with poor paralysis surveillance and high risk of polio https://polioeradication.org/news-post/explaining-environmental-surveillance/. As of 2014, 27 countries performed systematic reporting from environmental polio surveillance, another 13 had regional priorities tor adding systematic sites, and five countries performed environmental surveillance for polio virus as ad hoc surveillance or for research only https://pubmed.ncbi.nlm.nih.gov/25316848/. Several countries that had environmental surveillance on the "to do list" in 2014 had included it by 2020, resulting in a total of more than 550 environmental sites supporting polio paralysis surveillance (Global Polio Eradication Initiative annual report 2020 and semi-annual status updates. Geneva: World Health Organization; 2020).

There is, however, no proper standardization of methodologies used for sample site selection, sampling strategy, or further laboratory procedures before virus cultivation (Duintjer Tebbens et al., 2017).

There have been discussions on applying the well-defined sites and logistics of the polio environmental surveillance also for the environmental arm of the WHO One Health Survey (Tricycle project) (Årdal et al., 2021). Also, research led by the Dutch Public Health Institute (RIVM) and funded by the JPIAMR mechanism pilots this particular integration in one country, a project called TRIuMPH (Improving the TRIcycle protocol: Upscaling to national Monitoring, detection of CPE and WGS pipelines for One Health Surveillance), (https://www.rivm.nl/en/international-projects/triumph). In Pakistan, wastewater from the polio environmental surveillance sites have also been analysed for SARS-CoV-2, illustrating the feasibility of repurposing the environmental polio surveillance system for other pathogens (Sharif et al., 2021).

Norway does not perform environmental surveillance for polio. This is in line with advice on the (lack of) relevance of environmental surveillance in low-risk areas. However, the guideline points to an important exception in populations with documented high-level immunity to polio due to exclusive inactivated polio vaccine (as in Norway) that are frequently exposed to importation. This has been demonstrated in Israel, where

environmental surveillance demonstrated widespread transmission of imported poliovirus in the absence of clinical cases.

Although "transfer of AMR" potential by **migratory wildlife** is obvious, the use of such animals in a surveillance system for detection of AMR in the environment is challenging. There is no standardized system for this purpose, and monitoring and surveillance are particularly difficult because the animals can extend over large distances, especially migratory birds or mammals that seasonally move across continents or vast oceans. Opportunities to sample may be only brief at selected feeding or breeding locations (Mörner et al., 2002). International collaboration for following AMR in animals migrating between many different countries would be important.

Tabell AIII.1. Sustainable Development Goal 6.1: WHO/UNICEF JMP Drinking Water Quality Survey (MICS6)*

Link to protocol	https://mics.unicef.org/tools#data-collection

Main matrix	**Survey specific site selection**	**Regular microbiology target**	**Method**	**Frequency**	**Potential goal**	**Data repository****	**Suggested Norwegian setting**	**Suggested Norwegian data repository**
House-holds	At consumption[1]	*E. coli* number in 100 mL	Filtration[2], cultivation[3]	Once per year	Risk for transmission between compartments	washdata.org	Sampling under Norwegian Law (Drikkevanns-forskriften)	Vannverks-registeret (NIPH)

* Integrated water quality testing into household surveys, 2020. WHO/UNICEF Joint Monitoring Programme for Water supply Sanitation and Hygiene. Guidelines and templates for the 6th module of the Multiple Indicator Cluster Surveys programme (MICS6): https://mics.unicef.org/tools
** https://washdata.org/monitoring/drinking-water/water-quality-monitoring

[1]Randomly selected households. The measurer will ask the survey respondent for "a glass of water that members of your household would drink"
[2]Filtration equipment especially developed for field application
[3]Compact dry plates have been especially developed for MICS6, containing X-Gluc, and rehydrated by the sample. Samples are incubated in a special belt (to be worn around the waist) or in electric incubator. Results recorded after 24-48 hours.

Table AIII.2. Sustainable Development Goal 6.3.2: **UNEP Global Environment Monitoring System for freshwater quality (GEMS/Water)**

Link to protocol	https://www.unwater.org/app/uploads/2018/05/Step-by-step-methodology-6-3-2_Revision-2018-03-02_Final.pdf							
Main matrix	**Survey specific site selection**	**Microbiology target**	**Method**	**Frequency**	**Potential goal**	**Data repository**	**Suggested Norwegian setting**	**Suggested Norwegian data repository**
Ambient fresh water	Rivers Lakes Ground water	No microbiology target in core parameter (Level 1 monitoring)[1] *E. coli* and total coliform count at Level 2 monitoring[2]	Cultivation	Not assessed	Risk for transmission between compartments	https://gemstat.org/	NIVA	NIVA

[1]Level 1 parameters: dissolved oxygen, electrical conductivity, nitrogen, phosphorus, pH

[2]Level 2 parameters (optional): pathogens, biological approaches, modelled data, earth observations. *"However, where water bodies are used directly for drinking water without treatment, inclusion of microbiological parameters is highly recommended"* (citation from Step-by-step methodology/protocol), and specified in:

https://communities.unep.org/display/sdg632/Documents+and+Materials?preview=/32407814/38306559/CDC_GEMI2_TechDoc4_Level2_20200417.pdf#DocumentsandMaterials-Technical

Tabell AIII.3. Polio Environmental Surveillance

Link to protocol	https://polioeradication.org/wp-content/uploads/2016/07/WHO_V-B_03.03_eng.pdf

Main matrix	**Survey specific site selection**	**Regular microbiology target**	**Method**	**Frequency**	**Potential goal**	**Data repository****	**Suggested Norwegian setting**	**Suggested Norwegian data repository**
Human waste	Wastewater, open drains (source population 100,000-300,000)	Polio virus	Grab sample, virus cultivation	Not assessed	Proxy for AMR prevalence	National level	Commercial water laboratories	NIVA

* Integrated water quality testing into household surveys, 2020. WHO/UNICEF Joint Monitoring Programme for Water supply Sanitation and Hygiene. Guidelines and templates for the 6th module of the Multiple Indicator Cluster Surveys programme (MICS6): https://mics.unicef.org/tools
** https://washdata.org/monitoring/drinking-water/water-quality-monitoring

[1]Randomly selected households. The measurer will ask the survey respondent for "a glass of water that members of your household would drink"
[2]Filtration equipment especially developed for field application
[3]Compact dry plates have been especially developed for MICS6, containing X-Gluc, and rehydrated by the sample. Samples are incubated in special belt (to be worn around the waist) or in an electric incubator. Results are recorded after 24–48 hours.

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