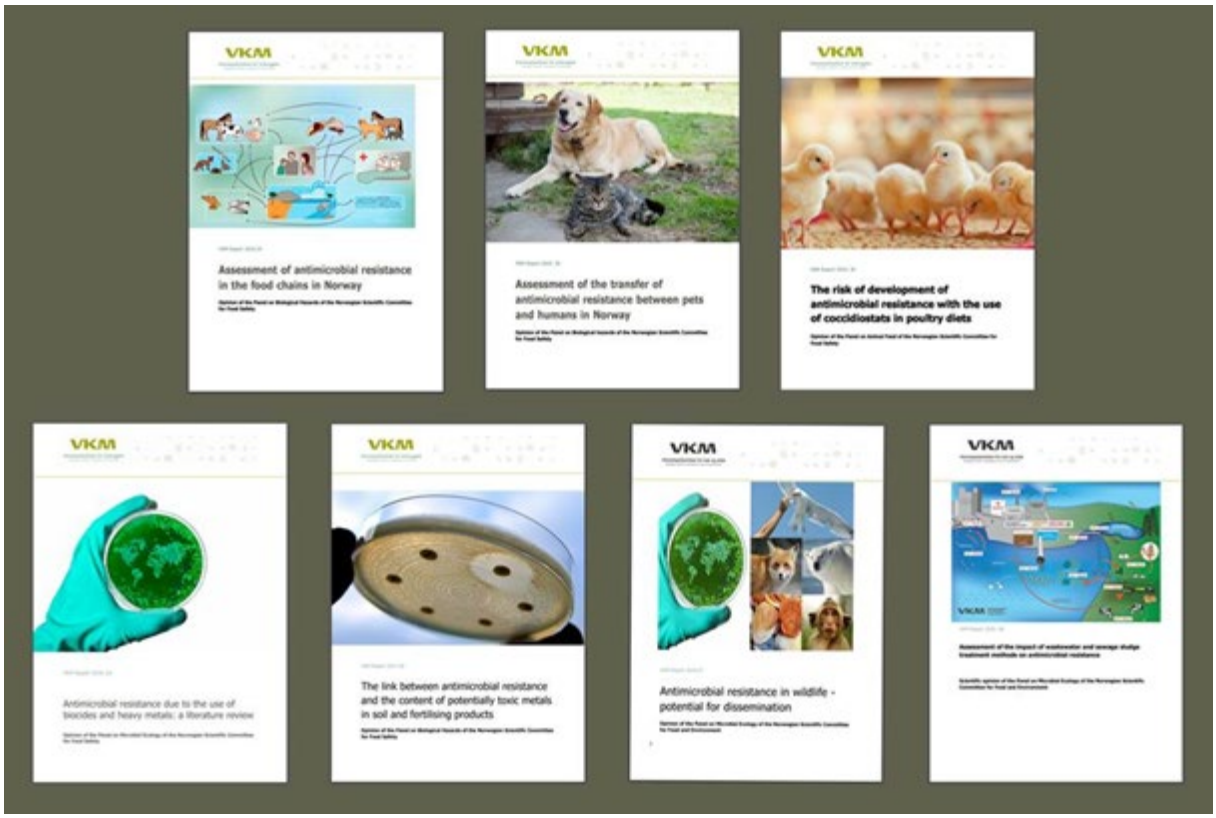
Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment




VKM Report 2020: 10

# Antimicrobial resistance (AMR) from an environmental perspective

A short summary of assessments prepared by VKM in the period 2015-2020.

**Antimicrobial resistance (AMR) from an environmental perspective - a short summary of assessments prepared by VKM in the period 2015-2020.**

Scientific opinion of the Panel on Microbial Ecology of the 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 answer the request from The Norwegian Environment Agency. The project group consisted of two persons, and a project leader from the VKM secretariat.

## 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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**Acknowledgment**

The project leader from the VKM secretariat, Siamak Yazdankhah, is acknowledged for coordinating the work.

**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

# Summary

The Norwegian Environment Agency (NEA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to summarize environmental aspects of antimicrobial resistance (AMR) in the assessments published in the period 2015-2020. The environmental aspects of resistance include three core dimensions related to the sources and dynamics of: i) antimicrobial-resistant bacteria (ARB), ii) antimicrobial-resistance genes (ARG) and their vectors, and iii) selective agents, including antimicrobials, heavy metals, and other compounds that may affect growth rates and dissemination of microbes. Here we provide a brief summary of these aspects in relevant assessments and highlight significant challenges in the field of AMR in the environment during 2020, as identified by the VKM Panel on Microbial Ecology.

Seven assessments developed during the period 2015-2020 were considered to be of relevance within the context of environmental aspects of AMR. Four of the published assessments did not focus specifically on AMR in the environment, whereas three of the assessments had a clear focus on AMR in various environmental compartments. These were assessments of: heavy metals and biocides, potentially toxic metals (PTM) in agricultural soils, and AMR in wildlife. Relevant aspects from all seven assessments have been extracted and discussed in the light of the 2020 request from the NEA.

From these assessments, the following significant challenges in the field of AMR in the environment today were identified:

The majority of published studies describing AMR in the environment remain descriptive and report on the point prevalence of specific resistance traits among relatively few samples. These data are thus fragmented, derived from a range of environments, and are often only descriptive. Moreover, the rationale for the choice of study population and sites is often lacking or explained in insufficient detail. This limits the studies' contribution to a holistic understanding of the microbial populations investigated and their broader dynamics. Most studies considered in the 2015-2020 assessments do not use a quantitative, longitudinal, or comparative approach.

In the experience of VKM, studies with the limitations described above often fail to provide a strong evidence base for risk assessments. Hence, previous assessments from VKM have been unable to deliver a full risk analysis due to the lack of quantitative and tempo-spatial dimensions in source material. A similar situation is evident regarding studies on antimicrobial agents and their environmental footprint. In most cases, data for Norwegian environments is scant and therefore most previous assessments were based on data obtained from other geographical locations, with variable relevance to Norwegian conditions.

A key conclusion from these assessments is that quantitative data, broadly describing a given environment and its dynamics over time, are rarely available. This means that relevant modelling and understanding exposure and resistance levels and how they vary over time, is seldom possible. Furthermore, the environment is rarely exposed to single genes, bacterial species, or selective agents, but mixtures of antimicrobials (and other pharmaceuticals and heavy metals), genes, and bacterial species that interact in complex pathways. The designs of studies considered in the 2015-2020 assessments have meant that it has not been possible to capture and address this complexity.

VKM recommends that more emphasis is put on experimental design, including longitudinal and representative spatial sampling, to improve the database for risk assessment. Moreover, consensus on relevant bioindicators (targeted/tracked organisms), achieved by closer coordination of efforts at an international scale, would be advantageous.

When considering resistance dynamics over time, VKM is of the opinion that it is essential to also focus on the selection in the environment investigated. That is, there should be detection and monitoring over time of those chemicals and conditions that positively select for resistance development and dissemination, in the same environment as the ARG and ARB are investigated. Keeping a parallel focus on resistance drivers will require further development of analytical tools that can determine the concentration range and dynamics of selective substances over time, their decay rates, their dispersal routes, and how human activities may affect and change their dynamics over time. It is possible that selection for AMR is not identified in the sampled environment, and, in that case, determination of concentration ranges would not be relevant. However, experimental studies can be designed that focus on addressing explicit hypotheses on how observed resistance traits have entered the sampled populations (e.g., through migratory animals including birds, human travel, weather conditions, etc.).

Our summary of assessments highlights the absence of appropriate, standardized methodology to determine how various chemicals act together in defining the overall selective conditions for the microbiome present in the given environment; for example, determining how various pharmaceuticals can have synergistic effects and how heavy metals and other compounds (e.g., pesticides, other pharmaceuticals) provide mixtures of chemicals with possible environmental effects. The ecological footprint of commonly used pharmaceuticals should be further reviewed as possible co-selectors for resistance development.

For a given environmental resistance trait, it is currently not possible to define whether the trait was naturally present prior to introduction of pharmaceutical antimicrobials, whether it was amplified or emerged due to directional selection, whether it emerged due to the global spread of mobile genetic elements (transposons/plasmids), by chance distribution, or is due to the occurrence of particular microbes. The presence of such a trait may reflect a combination of such mechanisms, and these can differ between sites, traits, and time. The factors underlying AMR dynamics are a key source of uncertainty that are rarely explicitly considered and tested in resistance studies. However, some genetic signatures may reduce scenario uncertainty. For instance, the identification of identical resistance traits (and combinations thereof – preserved synteny) suggests recent origins and dispersal through horizontal gene transfer (HGT) mechanisms. As directionality of resistance gene flow remains challenging to prove/document, the Panel highlights the need for a One Health approach. Studies that collect data that can establish directionality, dynamics, and the key pathways and sites of gene transfer may provide valuable information.

A relevant comparative basis for the panel´s current summary of assessments is the 2014 report "Antibiotikaresistens – kunnskapshull og aktuelle tiltak" developed by an interdisciplinary national expert group in 2014 (FHI, 2014). Considering the assessments indicated above, the panel is of the opinion that some progress has been made in our understanding of AMR in nature and the environment since 2014. However, the suggestions and conclusions of the FHI report remain valid. Although numerous new resistance-oriented studies have been published since 2014, the majority are point-prevalence investigations, providing a snapshot of the resistance situation in a sampled bacterial population in a

defined study site. Often the occurrence of a limited set of known resistance traits is described in a narrow set of bacterial species. The frequent and ongoing addition of such studies to the scientific literature provides more information, but does not fundamentally change the questions, uncertainties, and challenges presented by the environmental reservoirs of ARB, ARG, and resistance drivers in 2020.

The Panel recommends building coordinated international efforts, conducting studies that go beyond descriptive approaches, standardizing sampling and analysis regimes, ensuring data accessibility on the use and interactions of selective agents, and developing monitoring strategies that improve our understanding of longitudinal resistance dynamics. The Panel notes that in the absence of a robust theoretical/epidemiological framework, we are not in a position to backtrack and understand the evolutionary pathways that led to our current resistance situation. This is a source of uncertainty when assessing scenarios and predicting future effects of various activities.

# Sammendrag

I denne rapporten oppsummerer Vitenskapskomiteen for mat og miljø (VKM) ved faggruppen for mikrobiell økologi miljøaspekter ved antimikrobiell resistens (AMR), som fremkommer i vurderinger fra VKM publisert i perioden 2015 - 2020. De miljømessige aspektene ved resistens inkluderer tre hovedelementer knyttet til kilder og dynamikk: i) antimikrobielle resistente bakterier, ii) antimikrobielle resistensgener og deres vektorer, og iii) selektive midler, inkludert antimikrobielle midler, tungmetaller og andre forbindelser som kan påvirke vekstrater og spredning av mikrober. VKM peker også på sentrale utfordringer knyttet til antimikrobiell resistens i miljøet i 2020. Oppsummeringen er gjort på oppdrag fra Miljødirektoratet.

Sju vurderinger publisert i perioden 2015-2020, ble ansett å være relevante for miljøaspekter ved antimikrobiell resistens. Fire av vurderingene fokuserte ikke spesifikt på antimikrobiell resistens i miljøet, mens tre av vurderingene hadde et klart fokus på antimikrobiell resistens i ulike miljøer. Det gjaldt disse tre vurderingene: tungmetaller og biocider, potensielt giftige metaller i landbruksområde og antimikrobiell resistens i viltlevende dyr. Relevante aspekter fra alle de sju vurderingene er hentet ut og diskutert i lys av forespørselen fra Miljødirektoratet.

Med utgangspunkt i disse vurderingene identifiserte vi at vi i dag står overfor følgende utfordringer:

Flertallet av publiserte studier som beskriver antimikrobiell resistens i miljøet er fremdeles deskriptive, og rapporterer punktutbredelse av spesifikke resistenstrekk fra relativt få prøver. Dataene fremstår fragmentert, hentet fra ulike miljøer, og er ofte bare beskrivende. Det mangler ofte begrunnelse for valg av studiepopulasjon og studielokaliteter, eller begrunnelsen er utilstrekkelig forklart. Dette begrenser studienes bidrag til en helhetlig forståelse av de mikrobielle populasjonene som undersøkes og bredden i populasjonenes dynamikk. De færreste studiene som inngikk i vurderingene fra 2015 - 2020 har en kvantitativ, langsgående eller komparativ tilnærming. Etter VKMs erfaring, er datagrunnlaget fra studier uten kvantitativ, langsgående eller komparativ tilnærming ofte utilstrekkelig for helhetlige risikovurderinger. På grunn av mangel på kvantitative og tempo-spatiale dimensjoner i datamaterialet, har tidligere vurderinger fra VKM derfor ikke inneholdt en full risikoanalyse. En lignende situasjon er også tydelig for studier på antimikrobielle midler og deres økoskygge. I de fleste tilfeller er data fra norske miljøer i liten grad tilgjengelige. De fleste tidligere vurderinger ble derfor basert på data innhentet fra andre geografiske steder, som i varierende grad er relevante for norske forhold. En viktig konklusjon fra disse oppsummerte vurderingene er derfor at kvantitative data, som beskriver resistensdynamikk over tid, sjelden er tilgjengelige. Dette betyr at det ofte ikke er mulig med relevant modellering og forståelse av eksponerings- og resistensnivåer og hvordan de varierer over tid. Videre bemerkes det at miljøet er komplekst, hvor det vil forekomme blandinger av antimikrobielle midler (og andre legemidler og tungmetaller), gener og mangfold av bakterier i miljøet. Utformingen av studier vurdert i 2015 - 2020 fanger i liten grad opp denne kompleksiteten.

VKM anbefaler at det legges større vekt på eksperimentell design, inkludert langsgående og representativ romlig/geografisk prøvetaking, for å forbedre tilgangen til relevant datamateriale for risikovurdering. Videre vil det være en fordel med konsensus om relevante bioindikatorer (definerte/sporbare mikroorganismer) ved koordinering av forskningsinnsats på en internasjonal skala.

Ved vurdering av resistensdynamikk over tid, er VKM av den oppfatning at det er viktig å også fokusere på seleksjonsfaktorer i miljøet som undersøkes. Det vil si at det også bør være langsgående deteksjon og overvåking av kjemikalier og miljøforhold som positivt selekterer for resistensutvikling og spredning, i de samme miljøer som antimikrobielt resistente bakterier og antimikrobielle resistensgener undersøkes. Å holde et parallelt fokus på seleksjonsdrivere krever videre utvikling av analytiske verktøy som kan bestemme konsentrasjonsområdet og dynamikken til selektive stoffer over tid, deres nedbrytningshastighet, spredningsruter og hvordan menneskelige aktiviteter påvirker og endrer dynamikken over tid. Det kan skje at bestemte seleksjonsfaktorer for antimikrobiell resistens ikke identifiseres i et miljø hvor prøver tas og resistens påvises. I så fall vil ikke bestemmelse av konsentrasjonsområder være relevant. Eksperimentelle studier som fokuserer på eksplisitte hypoteser om hvordan de observerte resistenstrekk har kommet inn i de populasjonene hvor prøvene hentes fra (f. eks. gjennom trekkdyr, inkludert fugler, reiseaktivitet, partikkeltransport ved værforhold osv.), kan imidlertid fremdeles utformes.

Vår oppsummering av vurderingene fremhever fravær av egnet standardisert metodikk for å avgjøre hvordan ulike kjemikalier kan virke sammen i å skape de selektive forholdene for mikrobiomet som finnes i et gitt miljø. For eksempel, for å bestemme hvordan ulike legemidler kan ha synergistiske effekter og hvordan tungmetaller og andre forbindelser (f.eks. plantevernmidler, andre legemidler) gir sammensatte blandinger av kjemikalier med mulige miljøeffekter. Legemidler utover antibiotika bør vurderes videre for mulige effekter som co-seleksjonsfaktorer for resistensutvikling.

For ett påvist resistensgen i et miljø, er det oftest umulig å definere om genet var naturlig tilstede før innføring av farmasøytisk-produsertantimikrobielle midler, eller om det tilkom senere. For eksempel ved at det ble oppformert ved positiv seleksjon, innført ved spredning mellom ulike miljø, herunder som en del av mobile genetiske elementer, eller som en følge av variasjon i populasjonsstørrelsen til bestemte mikrober. Påvisning av et resistensgen i et miljø vil gjenspeile en kombinasjon av slike spredningsmekanismer. Disse mekanismene vil variere mellom miljø og over tid, og deres kompleksitet er en viktig kilde til usikkerhet. Påvisning av noen genetiske signaturer kan imidlertid redusere slik scenario usikkerhet. For eksempel vil identifisering av identiske resistensgener i konserverte kromosomale kombinasjoner antyde nylig opprinnelse og spredning gjennom horisontale genoverføringsmekanismer. En retningsbestemt flyt av resistensgener mellom miljø, er fortsatt utfordrende å dokumentere. Faggruppen understreker derfor behovet for en én helse-tilnærming. Videre studier som samler inn data som kan etablere retning, dynamikk og de viktigste veiene og stedene for resistensgenoverføring, kan gi verdifull informasjon.

Et relevant komparativt grunnlag for faggruppens oppsummering av vurderinger, er rapporten "Antibiotikaresistens– kunnskapshull og aktuelle tiltak"" utviklet av en tverrfaglig nasjonal ekspertgruppe i 2014 (FHI, 2014). Med tanke på vurderingene som er angitt ovenfor, er faggruppen av den oppfatning at det er gjort noen fremskritt i vår forståelse av antimikrobiell resistens i natur og miljø siden 2014. Forslagene og konklusjonene i FHI-rapporten er imidlertid fremdeles gyldige. Selv om det er publisert mange nye resistensorienterte studier siden 2014, er flertallet punktprevalensorienterte undersøkelser. Dette gir bare et øyeblikksbilde av resistenssituasjonen i en prøve fra en bakteriell populasjon fra ett definert miljø/sted. Ofte blir forekomsten av et begrenset sett med kjente resistenstrekk beskrevet i slike studier for et smalt sett med bakterielle arter. Den hyppige publiseringen av slike studier i den vitenskapelige litteraturen øker den totale informasjonsmengden men endrer ikke de fundamentale spørsmål, usikkerhet og

utfordringer som miljøreservoarene til antimikrobielt resistente bakterier, antimikrobielle resistensgener og resistensdrivere representerer i 2020.

Faggruppen anbefaler å bygge opp en koordinert internasjonal innsats, utforme studier som går utover beskrivende tilnærminger, etablere standardisere prøvetakings- og analyseregimer, sikre datatilgjengelighet om bruk og interaksjoner mellom selektive midler, og utvikle overvåkingsstrategier som forbedrer vår forståelse av langsgående resistensdynamikk. Faggruppen bemerker at det er behov for videre utvikling av et robust teoretisk/epidemiologisk rammeverk. Dette for å kunne identifisere og forstå de mekanismene som førte til vår nåværende resistenssituasjon og videre resistensutvikling.

# 1- Background

The National Action Plan against antibiotic resistance of the Norwegian Government for 2015-2020 highlights that antimicrobial resistance (AMR) should be considered from a holistic perspective, in which human health, animal health, and the environment interact. Although the use of antibiotics in people and animals, for the purpose of therapy and/or prophylaxis, are the major drivers in the development of resistance in bacteria, other factors may also contribute. Resistant bacteria in different environments, such as soil, water, sediments, and wildlife, may transfer their resistance traits, and hence facilitate resistance development, to bacteria of clinical relevance and to other previously susceptible commensal and non-pathogenic bacteria. The presence and dynamics of AMR in the environment are influenced by a variety of anthropogenic factors.

The development and dissemination of AMR in the environment can be divided into 3 strata: a. the release and stability of antimicrobial agents, b. the fate of antimicrobial-resistant bacteria (ARB) from humans and animal sources, and c. the potential for horizontal gene transfer (HGT) of resistance genes, as illustrated in Figure 1 below (modified after da Costa et al. 2013).

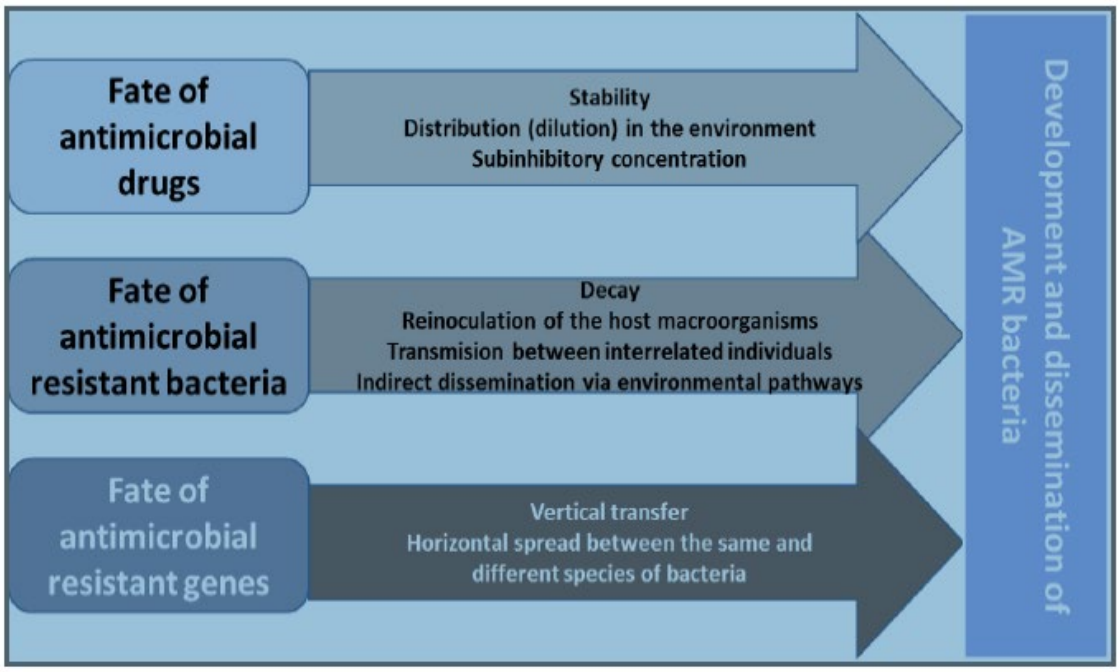


**Figure 1.** Development and dissemination of AMR (modified after da Costa et al. 2013).

In the strategy of the Norwegian Government, increased knowledge on the development of AMR is an important goal. The National Action Plan is based on the report "Antibiotikaresistens – kunnskapshull og aktuelle tiltak" that was compiled by a national expert group in 2014 (FHI, 2014).

Based on the National Action Plan, the Norwegian Scientific Committee for Food and Environment (VKM) prepared seven assessments regarding AMR on the request of the Norwegian Food Safety Authority (NFSA) and the Norwegian Environment Agency (NEA), in

the time period 2015-2020 (Appendix I). Assessments 1, 2, 3, and 5 were requested by NFSA, and assessments 4 and 6 were prepared on request from NEA. Assessment 7 was a joint request from NFSA and NEA.

**Terms of reference**
The Norwegian Environment Agency (NEA) requests Norwegian Scientific Committee for Food and Environment (VKM) to compile the status and challenges from relevant VKM reports prepared during the period 2015-2020, cf. Appendix.

NEA asks that, based on VKM reports on the topic of AMR in the period 2015-2020, VKM compiles a brief summary of what VKM sees as significant challenges in the field of AMR in nature and environment in 2020. The assignment includes ARB, ARG, and resistance drivers.

## 1-2- Definitions

### Environment

For the purposes of this report, **environment** is defined as the **natural environment**.

Manmade constructions are not included in the concept of natural environment in this report, which is thus defined as the biotic and abiotic components of plants, soil, sediments, water, air etc. with which an individual, population, or species comes in contact (Nielsen et al 2018).

### Antimicrobial agents

"Antimicrobial agents" is a general term for the drugs (antibiotics), chemicals, or other substances that either kill or inhibit the growth of microbes. The concept of antimicrobial agents applies to antibiotics, disinfectants, preservatives, sanitizing agents, and biocidal products. Antimicrobial agents include here antibacterial agents (antibiotics; both natural and synthetic), antifungal agents, disinfectant agents, and potentially toxic metals (PTM).

### Antimicrobial resistance

Antimicrobial resistance (AMR) is 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.

# 2- Method used to identify relevant assessments

Each assessment published within the defined period was scanned for search words regarding the environment (Appendix I). Information regarding AMR (antimicrobial agent residues, ARB, and ARG) in the environment were collected and evaluated from the environmental perspective, as outlined below for each of the 7 assessments considered relevant.

# 3- Results: assessments published between 2015-2020 considered

The following assessments were identified by the search words used and were further considered here:

## 3-1- Assessment of antimicrobial resistance in the food chains in Norway (Yazdankhah et al., 2015).

**Purpose**
This report focused on AMR in food chains in Norway, and the probability of exposure of people to AMR via these food chains.
Questions related to the environment were not a part of the terms of reference of the assessment.

**Summary of conclusions according to the 2015 terms of reference**

The clinical AMR crisis has focused attention on all uses of antimicrobial agents, including in human medicine, veterinary medicine, and agriculture and aquaculture. AMR is considered the greatest challenge to healthcare in the 21st century, and there is increasing concern and debate about the roles played by food-production chains as reservoirs and disseminators of AMR.

Some conclusions or major findings of the 2015 assessment:

- The probability of transfer of AMR from cattle, milk/milk products, fish, seafood, and drinking water was assessed to be **negligible**.
- The probability of transfer of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) from live pigs to humans was considered to be **non-negligible**, and the probability of transfer from pork to humans was assessed to be **negligible**.
- The probability of transfer of ESBL/AmpC-producing Enterobacteriaceae[1], quinolone-resistant *E. coli*, and their respective corresponding genes from live poultry and poultry meat was considered as **non-negligible**.
- Processing of food, such as cooking or preservation, can reduce the number of bacteria in the products and thus decrease the transmission of ARB from food to humans.
- Most studies were designed to determine point prevalence. Due to insufficient quantitative data, it was impossible to calculate the relative importance of ARB originating from food chains as compared with the total load of bacterial resistance to which humans are exposed. Thus, the impact on human health from ARB present in food chains was not estimated.

[1] Resistance in Gram-negative bacteria to extended-spectrum cephalosporins, like cefuroxime, ceftazedime, and cefotaxime, has been developing over two decades. It is most often caused by extended-spectrum β-lactamases (ESBLs) (class A, or ESBLA), but may also be conferred by plasmid-mediated AmpC-type enzymes (Class C, called ESBLM or pAmpC). Hyper-production of AmpC-type enzymes due to chromosomal mutations can mediate resistance to cephalsosporins.

### Summary of uncertainties

The conclusions of the assessment were associated with a number of uncertainties. Bacteria are living organisms that are under continuous evolution, and are able to adapt rapidly to changing living conditions. The 2015 report is an assessment of the current situation at that time, regarding development and dissemination of ARB and their resistance genes in the food chain. This situation changes continually as the bacteria continue to adapt, and new variants of bacteria emerge and spread. Such bacterial changes may also affect the prevalence and probability of transfer of resistance to specific antimicrobials. Furthermore, identifying animal-to-human transmission routes of AMR has yet to be fully developed, given the current lack of understanding of the complex genetic interactions and dissemination pathways between commensal and environmental bacteria.

### Summary of data gaps

Lack of systematically collected quantitative data made it difficult to reach firm conclusions regarding the probability of AMR transmission from food to humans in Norway. Similarly, ranking the probabilities with regards to their relative importance was largely not possible with the data available.

A lack of knowledge regarding the diverse reservoir of AMR in the environment, animals, and food reservoirs was identified. Furthermore, data on the routes and frequencies of transmission of AMR from live, food-producing animals and foodstuffs of different origins to humans, then to the environment, and vice versa, were lacking.

A need for additional data regarding antimicrobial agents' residues, ARB, and ARG in the environment originating from feed and food-producing animals, food-processing, and food was identified.

## Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

The environmental aspects and large footprint of the food chain was not the focus of this VKM report (Yazdankhah et al., 2015). Hence, we have not identified specific datasets in the report for further consideration.

Some of the knowledge gaps and uncertainties identified in the 2015 report are nevertheless still relevant when considering bacterial populations present in natural environments today. These have been further described in the general sections "Uncertainty" and "Knowledge gaps".

Taking a One-Health perspective, we note the large environmental footprint of bacteria shed from the gastrointestinal tract (GIT) of food-producing animals and the environmental effects of such faecal/manure sources - including the proportion containing resistance determinants. Moreover, many antimicrobial agents used in food-production animals will eventually end up in wastewater, sludge, or animal waste, from where they have the potential to reach new environments (Kummerer et al., 2000). Sewage sludge and animal waste are used as fertilizing agents in the production of food crops. Although recycling of nutrients is recommended for environmental and agricultural reasons, this also means antimicrobials and ARB can follow the same dispersal routes, if appropriate treatment before application is not implemented.

## 3-2 Assessment of the transfer of antimicrobial resistance between pets and humans in Norway (Wasteson et al., 2015).

### Purpose

This report focused on transmission of bacteria with AMR between pets and humans, and vice versa, and an assessment of the consequences that this may have on public health and animal health in Norway.
Questions related to the environment were not a part of the terms of reference of this assessment.

### Summary of conclusions according to the 2015 terms of reference

The prevalence of AMR among certain bacteria of the normal enteric microbiota in dogs and cats can serve as indicators for selective antimicrobial pressure in various populations. These bacteria may form a reservoir of transferable ARG from which AMR can spread to other bacteria, including those responsible for infections in animals or humans, or to environmental bacteria. Some conclusions or major findings:

- Increasing amounts of antimicrobials are used for treatment of pets, including numerous substances used in human medicine.
- The main bacteria of concern include *Staphylococcus pseudintermedius* and *Escherichia coli*, as well as other organisms of clinical importance in human medicine, such as MRSA and Enterobacteriaceae spp.
- Transmission of such organisms occurs between pets, owners, and veterinary staff. Pets can act as reservoirs of these bacteria and their occurrence can impact on the use of antimicrobials in human medicine.
- A 19 % increase in sales of antimicrobials marketed for companion animals occurred in Norway between 1995 and 2014. This increase was mainly accounted for by penicillins, and approximately 87 % of penicillin products sold for use in companion animals during 2014 was as a combination of amoxicillin and clavulanic acid.

A major challenge in the prediction of AMR development was the limited ability to differentiate between the observed short-term effects of individual cases and transfers, and the more long-term effects created by emerging patterns of AMR. Thus, one plausible description of the current situation (in 2015) was that the use of antimicrobials in dogs could be of marginal importance in the short-term, but may have significance through environmental contamination over time.

### Summary of uncertainties

The risks attributable to transmission of ARB or resistance determinants between household pets and humans were difficult to quantify due to a multitude of knowledge gaps, mainly because most information on transmission of microbes by household pets is derived from single case reports.
There is therefore considerable scenario uncertainty associated with bacterial evolution.

### Summary of data gaps

A lack of data regarding the broad reservoir of AMR in the environment, pet animals, and human populations was identified. Furthermore, there was lack of data regarding the routes

and frequencies of transmission of AMR from pet animals to humans and the environment, and vice versa.

A diverse, and largely unexplored, reservoir of ARG was considered likely to be present in non-pathogenic bacteria in the environment or in commensal microbes. Studies using metagenomics have highlighted the unappreciated diversity of AMR genes in the human microbiome, and genes that had not been described previously have been identified (Penders et al., 2013). Thus, the potential reservoir for resistant microbes and/or ARG in the human, animal, and environmental microbiomes remain to be explored. Interspecies microbial transmission is complex, and its implication is not well understood.

A lack of data regarding international travel, which enables interactions between the microbiota of both humans and pets with microbes from travel-related environments and populations, was identified. Although the consequences of these changes (migration) in the microbial genetic pool are difficult to predict, migration-facilitated dispersal of resistance traits could create opportunities for HGT to other bacteria.

### Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

The environmental aspects and larger footprint of the microbiota of pets were not the focus of this VKM report (Wasteson et al., 2015). However, the authors discussed how residues of antimicrobial agents in the urine and faeces of pets may contribute to the development of AMR in environmental bacteria. In urban areas with many pets, AMR is likely to occur in bacteria in the environment, even if they are shed by only a low proportion of pets. Most pets that are allowed outdoor access will both be exposed to, and shed, bacteria and bacterial genes by defecating, urinating, eating, licking, and salivation. For direct contact between dogs, and between dogs and humans, skin-skin and mouth-skin contact are relevant. AMR in bacteria in the skin, oral cavity, and throat is of interest. If ARB are shed in the urine or faeces they could be transferred to animals in the environment, including wildlife (such as birds and rodents).

## 3-3 The risk of development of antimicrobial resistance with the use of coccidiostats in poultry diets (Nesse et al, 2015)

### Purpose

This report focused on whether, and potentially how, the use of coccidiostats in feed for poultry could contribute towards an increased occurrence of ARB. Whether there are differences between the various coccidiostats and the status of use of those approved in Norway, compared with those authorized for use in the EU, regarding potential development of AMR was also evaluated.

Questions related to the environment were not a part of the terms of reference of this assessment.

## Summary of conclusions according to the 2015 terms of reference

Development of resistance in coccidia to all eleven coccidiostats considered was found to have been described in the scientific literature, but the prevalence of resistance was unknown. Cross-resistance between various ionophore coccidiostats has also been shown (i.e., development of resistance to one ionophore may result in the coccidia becoming resistant to another ionophore).

Development of resistance against ionophores has also previously been observed in bacteria. In the Norwegian surveillance programme, NORM-VET, during the years 2002 - 2013, between 50 - 80 % of the tested flocks had narasin-resistant faecal enterococci; these bacteria are part of the normal intestinal microbiota. However, *Clostridium perfringens,* which is pathogenic, was not shown to develop resistance against ionophores. Cross-resistance in bacteria to more than one ionophore has been reported. In addition, some data indicated an association between narasin and resistance to the antibacterials bacitracin and vancomycin.

## Summary of uncertainties

The assessment considered it likely that horizontal transfer of resistance against coccidiostats may occur, as HGT of a large variety of other antibacterial resistance genes is known to occur in bacteria. However, only one scientific study was identified at that time that described such transfer, reporting HGT of resistance against narasin *in vitro*. As none of the coccidiostat-resistance genes had been described in scientific literature, the extent to which these genes are located on transferrable gene elements was not known. Consequently, the frequency of such gene transfer was also unknown.

An association between resistance against narasin and bacitracin, and between narasin and vancomycin, has been reported. In addition, two publications described the results of laboratory studies that may support these data; one reporting on narasin resistance and vancomycin resistance in enterococci, and the other on lasalocid resistance and bacitracin resistance in *Clostridium aminophilum*. However, as the amount of data was very limited, and the level of uncertainty was high firm conclusions could not be reached. Characterization of ARG(s) against narasin could elucidate a possible link between resistance against narasin and resistance against bacitracin or vancomycin in enterococci.

## Summary of data gaps

At the time of this assessment, little was known regarding the consequences of human exposure to coccidiostat-resistant bacteria or to coccidiostats. In addition, there was little information indicating whether such bacteria could colonize the human body, either transiently or permanently. Furthermore, information on the probability of exchange of ARG from such bacteria to bacteria of the natural human microbiota or to pathogens was limited. In addition, no information was available on the levels of exposure (e.g., the amount of coccidiostats and their metabolites), or the period of exposure necessary for the various bacteria to give rise to resistant variants. As coccidiostats are not used to treat infectious diseases in humans, resistance concerns are related to possible cross- or co-resistance with antibacterials considered important in human medicine. Such resistance has not been identified to date.

The coccidiostat narasin also inhibits or kills various bacterial species, and it has been shown that bacteria in the normal microbiota of poultry (i.e., enterococci), develop resistance to

narasin. Although not used to treat infectious diseases in humans, data may indicate an association between narasin resistance and resistance to antibacterials considered important in human medicine. However, data addressing this question are scarce. Furthermore, genes conferring resistance to narasin have not been described in scientific literature.

The use of coccidiostats with an antibacterial effect in poultry feed can create a reservoir of ARB that could, theoretically, spread to humans by direct contact with animals and manure, or through the food supply. Furthermore, bacteria of the human normal microbiota could, theoretically, develop resistance from exposure to coccidiostats. Farmers and other workers in the poultry-production chain may be exposed to in-feed coccidiostats when handling feed and manure.

**Updated information regarding use of coccidiostats in Norway in 2020**
According to data obtained through annual reports from NFSA, total sales of coccidiostats as feed additives in Norway in 2014 was 13,772 kg (12,409 kg narasin and, 1313 kg mononsin) (NORM-NORM/VET 2014). In February 2015, the Norwegian poultry industry launched a project aimed at phasing out the use of narasin as a feed additive in broilers, and this goal was achieved in June 2016 (NORM-NORM/VET 2018). Narasin may still be used for control of necrotic enteritis (*C. perfringens*), and 52 kg of narasin was used for this purpose in 2016, according to NORM-NORM/VET (2018). Monensin is also still used in Norwegian turkey production, but its use has been reduced from 1313 kg (2014) to 820 kg in 2016 (NORM-NORM/VET-2018).

There is currently no indication of cross-resistance between narasin and monensin, and monensin resistance has not been associated with vancomycin resistance in bacteria. It is therefore not obvious why a large proportion of the *Enterococcus faecium* population of turkeys is narasin resistant, nor does it explain why vancomycin-resistant Enterococci (VRE) were absent from turkey samples in 2018.
Although a clear-cut conclusion cannot be drawn, it is possible that the interventions initiated by the poultry industry in 2015 – 2016 contributed to this reduction in VRE in the Norwegian poultry population (NORM-NORM/VET 2018).

## Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

A direct effect on the natural environment was not identified in this assessment. However, poultry manure is a valuable fertilizer and poultry manure-based compost or poultry manure is either applied directly to agricultural fields by farmers, stored for later land application, or transported for further treatment (composting, heating etc.) and preparation for commercial fertilizers. Poultry manure-based fertilizers are used in agriculture, as well as gardens and kitchen gardens.

Several studies have confirmed residual coccidiostats in poultry excreta and manure. The concentration levels depend on various factors, including the age and treatment of the manure, the properties of the coccidiostats, and the environmental conditions. The risk that workers handling manure are exposed to coccidiostats is greater for manure that is fresh or has undergone short-term storage than for composted manure. A study performed by Ghent University indicated the residue levels that are expected in excreta and manure during storage, composting, and transfer to certain crops (see references within Nesse *et al.*, 2015). However, due to differences in practical aspects and the high variation in decay and dispersal, the concentration levels to which the workers might be exposed may be lower or

higher than reported in this study. The challenge of sampling of non-homogenous matrix, such as manure, might influence the results, and should be considered during interpretation of data.

## 3-4 Antimicrobial resistance due to the use of biocides and heavy metals: a literature review (Tronsmo et al., 2016)

### Purpose

This report compiled available literature and relevant information on biocides and heavy metals, and their possible roles in the development of AMR. Substances that were of most relevance to analyze further in relation to the presence and increase in AMR were identified, with focus on the Norwegian environment.

Questions related to the environment were an important part of this assessment.

### Summary of conclusions according to the 2016 terms of reference

The production and usage volumes for several biocides and heavy metals are several orders of magnitude higher than those of antimicrobial agents used in therapy and prophylaxis in human and veterinary medicine, and their area of application is also considerably greater.

The potential of different biocides and heavy metals to induce AMR and cross or co-resistance in bacteria was defined as "highly likely" (expected to occur in most circumstances), "likely" (could occur in many circumstances), and "unlikely" (could occur in some circumstances) as below:

1- Highly likely: the heavy metals copper, zinc, and cadmium.
2- Likely: phenols, especially triclosan, surface-active agents, especially quaternary ammonium compounds (QACs), and the heavy metals arsenic and mercury.
3- Unlikely: aldehydes, biguanides, organic acids, inorganic acids, antimicrobial dyes, diaminides, and silver.

### Summary of uncertainties

Uncertainties regarding the ability of biocide-resistant and/or heavy metal-resistant bacterial strains to colonize humans or animals were also identified, including to the extent to which this occurs. The ability of ARG to be transferred from resistant strains to resident bacterial species in the environment was also an uncertainty.

### Summary of data gaps

There was a lack of knowledge regarding the diverse reservoir of AMR in the environment, including in soil, sediments, water, air, wild plants, and animals that are impacted by biocides and heavy metals. The assessment was not able to gather sufficient data on the amount of the different biocides and heavy metals that end up in the Norwegian environment, nor the extent to which exposure to such substances, alone or in combination with other antimicrobials, may result in development of AMR in microbial communities. Furthermore, limited data were available regarding use/misuse/presence of biocides and

heavy metals in consumer products. Knowledge regarding the development of AMR in bacteria due to use of biocides or heavy metals in cosmetic products was lacking.

Detailed data on the use of biocides in Norway, along with their environmental levels, was not readily available. Without these data, estimating the selective pressure that could potentially select for increased AMR was challenging. In contrast, the environmental levels of heavy metals in soil, sewage, and sediments were monitored at regular intervals and these data were used for exposure considerations. However, the presently used methods for determination of AMR in environmental samples was, and is, primarily based on culture studies (± antibiotics) or on the presence of ARGs (by qPCR or sequencing). As these methods do not fully capture the potential for co-selection with biocides or heavy metals, there is uncertainty in interpreting the data.

### Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

The 2016 assessment indicated the unresolved role of heavy metals in selecting for resistance in bacteria, and the possible impact on therapeutic options. Moreover, the relevant timeframe was not identified, and standardized methodology to investigate mixture toxicology and resistance development has yet to be developed. Data availability is of concern as heavy metals are used according to different regulations. In contrast to most other antimicrobial agents, heavy metals do not decay, and therefore different accumulation and decay/dispersal rates and models are needed.

Understanding the release of potentially resistance-driving chemicals to environments where bacteria, antibiotics, ARG, and co-selecting environmental factors (like heavy metals and or biocides) meet, for example in urban and agricultural sewage and in industrial wastewater (in particular pharmaceutical and food production), is therefore of high importance. The ecological role of disinfectant-resistant and heavy metal-resistant bacteria of environmental origin, with concomitant resistance against antibiotics, in impacting human bacterial populations is unclear.

Both heavy metals and biocides may induce resistance in bacteria in the environment, of which some may confer cross-resistance to antimicrobials.

## 3-5 The link between antimicrobial resistance and the content of potentially toxic metals (PTM) in soil and fertilising products (Wasteson et al., 2017)

### Purpose

This report focused on whether the content of arsenic (As), cadmium (Cd), chromium (CrIII + CrVI), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) in soil and fertilising products that are relevant for Norway play a role in the development, spread, and persistence of bacterial resistance to these elements, as well as cross- or co-resistance to antimicrobial agents.

Questions related to the environment were an important part of this assessment.

## Summary of conclusions according to the 2017 terms of reference

Investigation of PTM-driven co-selection of AMR in environments impacted by agriculture and aquaculture should focus especially on Cu and Zn, which may be added to animal feed, and on Cd because of its high bioavailability and toxicity. The spread of resistance towards the PTM evaluated in this assessment involves cross- and co-resistance to antimicrobial agents used in prophylaxis and therapy in animals and people. Most important are those cases where toxic-metal resistance is coupled to resistance towards highly important and critically important antibiotics[2]. This has been described in some of the published articles included in this assessment. We do not fully understand the mechanisms behind persistence of AMR, and removing drivers for development and spread of resistance may result in a decrease in the levels of AMR, but not necessarily full disappearance.

## Summary of uncertainties

Several sources of uncertainty were identified. Knowledge on the abundance of ARG (resistome) in different environmental sites and samples is not readily available or data have not been systematically collected. Therefore, in general, it was difficult to distinguish between the natural resistome and an elevated abundance of ARG in the different environmental samples due to anthropogenic exposures. The naturally occurring background AMR in environmental bacteria was found to complicate the estimate of the effect of PTM exposure on development of resistance.

Baseline data regarding PTM concentrations and PTM-resistant bacteria in the environment were limited. The lack of baseline data and studies created uncertainty and limited prediction of the long-terms effects of PTM in fertilizing products, regarding development and persistence of AMR in the environment.

## Summary of data gaps

A lack of knowledge regarding links between the concentrations of PTM in fertilizing products and soil and the development of resistance in bacteria was identified. Data regarding the routes and frequencies of transmission of AMR from bacteria of environmental origin to bacteria of animal and human origin were lacking in the articles available for review in this report. In the absence of such data, estimating the probability of development, transmission, and persistence of PTM resistance in the Norwegian environment was difficult. More research was needed to explain the relationship between development of resistance against PTM in bacteria and resistance toward antimicrobial agents.

[2] Each antimicrobial agent (or class) has been assigned to one of three categories of importance on the basis of 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.
The 3 categories were:
Critically important: antimicrobials that meet both criteria.
Highly important: antimicrobials that meet 1 of the 2 criteria.
Important: antimicrobials that do not meet either criterion.

### Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

The additive effect of toxic metals in fertilising materials should be considered from a long-term perspective, as these metals accumulate in the environment. In contrast, most other compounds with antimicrobial effects are organic in origin and decay over time.

It is currently not possible to determine the effects of partly unknown and variable levels and forms of PTM on the development, dissemination, and persistence of AMR. However, areas of intensive agriculture may be regarded as "hot spots" for interactions between bacteria of environmental, animal, and human origin, and PTM. The bioavailability of PTMs should be considered in this context.

It is noted that the veterinary use of Zn in Norway (in feed or for treatment) may have changed in recent years, and exposure scenarios should be revised accordingly (Sele et al., 2019).

## 3-6 Antimicrobial resistance in wildlife - potential for dissemination (Nielsen et al., 2018).

### Purpose

The purpose of this report was to elucidate the possible role of wildlife in dissemination of AMR in general terms, but with emphasis on the environment and wildlife of relevance to Norway. In this assessment, wildlife was defined as feral animals, captive wild animals, and wild animals. Wild animals were defined as animals with a phenotype that has not been affected by human selection and living independently of direct human supervision or control (i.e., living and roaming freely in their natural environment, and not domesticated or tamed).

Questions related to the environment were an important part of this assessment.

### Summary of conclusions according to the terms of reference

The selective pressure generated by the use of antimicrobial agents in human and veterinary medicine, livestock and plant production, as well as in aquaculture, was found to be the major source of high-levels of AMR in bacteria. Sharing of common habitats and water resources could result in transfer of ARB between wildlife, food-producing animals, and humans. Bacterial populations with various transferable AMR traits were found to be routinely reported in wildlife. Moreover, wildlife is a well-established source of AMR bacteria entering the food chain, both in meat and in foods of plant origin. The relative importance of such reservoirs and transfer routes of AMR in comparison with other sources leading to AMR development in pathogenic bacteria was not determined in the assessment.

Birds, particularly migratory birds, were considered to have the highest dispersal capacity for ARB due to their biannual migration patterns between countries and even continents. The role of omnivorous species was also highlighted as they often feed on anthropogenic waste and live near human communities and farms. Such species (e.g., rodents) are often ubiquitous and have the potential to act as a major link between wildlife, domestic animals, and humans. Furthermore, small anthropophilic prey species, such as mice and rats, form a

bridge between humans/domestic animals and their predators, including birds, some of which have seasonal migration patterns.

### Summary of uncertainties

A range of uncertainties on the understanding of the probability of development and dissemination of AMR from wildlife was identified. Many of these were due to data gaps, lack of understanding of relevant timescales, and an absence of a quantitative approach and broader theoretical framework that could have guided experimental design in the studies reviewed. Although many point-prevalence studies were available, few had been designed to establish directionality of resistance transfer. Very few included repeated sampling of populations over time. Other central uncertainties that influenced the conclusions of the assessment were the limited description of sources of antimicrobial agents in the environments of the wildlife populations under investigation and the lack of information on the effects of such substances in mixtures and at sub-inhibitory levels.

### Summary of data gaps

Many of the studies included were descriptive, with very small sample sizes and lacking quantitative and longitudinal perspectives. The lack of quantitative focus, as well as limited information on the population structure of the populations sampled and on the genetic basis for most of the resistance traits described in the studies, limited the understanding. Furthermore, the assessment was not able to identify exposure pathways or to infer the evolutionary trajectories of particular resistance determinants due to the absence of the relevant information in the source studies.

## Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

The environmental focus of the 2018 assessment is relevant for addressing the NEA's 2020 mandate, as it provides an overview of AMR in wildlife, a key component of the environment. The 2018 assessment concluded that: ARG (identified in clinics) are widespread in wildlife, although at highly variable frequencies; some form of AMR can be identified in almost all sampled wildlife. The genetics of such ARG suggest a shared gene pool and horizontal transfer of genes and/or bacterial vectors, the latter further facilitated by the fact that many warm-blooded wild animals carry similar species of gut bacteria as humans. The studies considered in the 2018 assessment were generally skewed towards detecting known, clinically relevant ARG in common bacteria of human relevance.

Most of the studies considered for that assessment were descriptive, point-prevalence investigations, with small sample sizes, and lacking quantitative and longitudinal perspectives. In addition to the lack of quantitative focus, data gaps and limited information on the larger genetic context for most of the resistance traits described in the studies were noted. This made it difficult to understand the extent to which the identified ARG were horizontally mobile and under what time perspective. That is, the extent to which these genes were part of larger transferable mobile units that could reestablish in new host bacteria, or were limited in their temporal and spatial mobility, could not be determined.

Uncertainties are listed in the generic "Uncertainty" chapter. The assessment emphasized the lack of harmonized methods necessary for comparing studies from different systems.

Moreover, a theoretical framework, which can be used to structure studies of complex genetic interactions that occur in environmental bacteria present in different species and host environments, was not in place.

In conclusion, the 2018 assessment provides an overview of the occurrence and diversity of ARB in a broad range of wildlife, based largely on point-prevalence studies. It was not possible to determine past exposure pathways nor to infer the evolutionary trajectories of the particular resistance determinants reported.

Further studies were suggested, and efforts at the international level to ensure uniform sampling strategies would be of value. Furthermore, a shift in practice from scattered point-prevalence studies, along with the development of a theoretical framework that can guide experimental design, would improve the value of future investigations.

## 3-7 Assessment of the impact of wastewater and sewage sludge treatment methods on antimicrobial resistance (Wasteson et al., 2020, in preparation)

### Purpose

The purpose of this assessment was to expand the 2009 VKM report "Risk assessment of contaminants in sewage sludge applied on Norwegian soils" to include issues related to AMR. The expansion specifically included the impact of wastewater (WW) and sewage-sludge treatment methods used in Norway, on the fate and survival of ARB and the fate of ARG and drivers for resistance (such as antibiotics, antifungal agents, heavy metals, disinfectant agents, etc.).

Questions related to the environment were an important part of this assessment. However, the report focuses mainly on methods for treatment of WW in wastewater treatment plants (WWTPs).

### Summary of conclusions according to the 2020 terms of reference

Some of the preliminary conclusions from this recent assessment are summarized below:

There were no strong indications that there is significant enrichment of ARB in WWTPs operated under European conditions, which, generally, are the conditions used in Norway.

During wastewater treatment (WWT), bacteria largely adhere to particles that are aggregated and precipitated to form sludge. The mandatory hygienisation of sludge kills a large proportion of these bacteria, notably all thermosensitive faecal bacteria.

The outcome of many treatments will limit the extent to which ARB of faecal origin are transferred to the food-production chain. Soils contain a pool of both natural and sludge-derived AMR. The contribution of sludge to this pool is probably temporally limited to the period immediately after soil amendment with sludge.

Hospital WW contains more ARB, ARG, and antibiotic residues than municipal sewage, but the difference is not large for ARB and the impact may be minimal in large WW systems. In smaller WW infrastructures, a hospital or similar institution may have a higher impact on the

EWW from the WWTP; in such instances, local treatment of WW at the hospital could be advantageous.

Due to the high concentrations of ARB and ARG (and also pathogens) in sewage, risks from sewage pipe leakages are of concern. Intrusion of contaminated water into the drinking-water distribution system should be of concern.

WWTPs in Norway (and the rest of the world) are generally not designed for removal of AMR (ARB and ARG). Membrane processes could be a promising option for increasing such removal rates.

Treatment of sludge significantly reduces the number of faecal indicator bacteria. However, due to the limited amount of studies looking at the ARB fraction in specific species and/or genera, it is impossible to reach a firm conclusion regarding the potential selection of phenotypic resistance during sludge treatment.

There are indications that conventional sludge-treatment methods are not particularly efficient at removal of ARG or other nucleic acids.

### Summary of uncertainties

Insufficient knowledge on the consequences to human/animals following exposure to residues of antimicrobial agents in EWW and sludge in the environment was noted. There is remaining uncertainty on the potential for survival and establishment of ARB in the environment and whether this would result in any subsequent effects on the microbiological balance in the ecosystem.

WWTPs and sludge-treatment facilities are relatively heterogenic regarding technique and volume, and detailed knowledge on the effect of different WW treatment technologies (physical, chemical, biological) on their ability to reduce residues of antimicrobial agents and their metabolites, ARB, and ARG. There are also large variations within a WW system regarding flowrates and influent types. This results in variation, and thus the results of studies conducted at a given WWTP cannot be extrapolated to all WW and WWTP.

### Summary of data gaps

There is currently a lack of harmonized methods and protocols, and therefore it is difficult to compare studies from different WWTPs. More research is needed on the cocktail effect of antimicrobial agents (antibacterial, antifungal, PTM, disinfectant agents) in the development of AMR in bacteria in WW. More data are needed to identify and quantify the sources, occurrence, and transport of antimicrobial residues (antibacterials, antifungals, disinfectants, PTM, and other substances), ARB, and ARG to environmental media like water, WW, and other environmental compartments to which human/animals are exposed.

## Environmental aspects that have been identified to be of relevance to NEA's 2020 mandate

The majority of the faecal microbiota in sewage systems is of human origin, as most livestock faeces end up as manure, which is used according to current guidelines. However, sewage systems also drain industrial activities, such as slaughterhouses and dairies, and this means that microbiota of animal origin may also end up in the public sewage systems. The

most prominent routes for environmental dissemination of AMR via WW are release of EWW and application of sludge to agricultural land.

Due to lack of comparative data, variability, measurement uncertainties, and the limited availability of standardized methods, it is difficult to determine the relative importance of AMR originating from WWTP to the total load of bacterial resistance to which humans and animals are exposed.

# 4-Significant challenges in the field of antimicrobial resistance in nature and the environment in 2020.

Of the seven relevant assessments from VKM that were produced in the period 2015-2020 and are considered here, four did not focus specifically on AMR in the environment. The other three assessments had a definitive focus on AMR in various environmental compartments (heavy metals and biocides, PTM in agricultural soils, and wildlife). The relevant conclusions from these have been described above in the light of the 2020 mandate. In most cases, the assessments are based on limited data material for Norwegian environments. This means that the assessments are often based on data collected from other locations, which may be of variable relevance to Norwegian conditions. For instance, heavy metal use and disposal practices may vary between countries, regulations are usually nationally defined, and wildlife composition and behaviour also differ between countries and regions. Thus, most of these assessments with an environmental AMR component provide conclusions that are based on fragmented data collected by researchers with different objectives, research questions, and study areas.

A key observation in all seven assessments is that quantitative data, broadly describing a given environment and its dynamics over time, are rarely available. This absence has limited the opportunities to model and understand exposure levels, and, hence, to conduct a full risk analysis.

To enable exposure assessment (of AMR drivers, genes, and vectors/bacteria), more emphasis should be directed towards experimental design and longitudinal sampling, with a consensus on relevant bioindicators (targeted organisms) and coordination of efforts at the international scale.

It is also essential that AMR selection in the environment being investigated is considered. That is, studies that determine and quantify those chemicals and conditions that positively select for resistance development and dissemination within the same environment that the ARB and ARG are investigated are more likely to provide relevant information. This requires an understanding of the concentration ranges and dynamics of the selective substances over time, their decay rates, their dispersal routes, and how human activities could affect and alter their dynamics.

However, within this context, it is also important to highlight the lack of appropriate, standardized methodology for determining how various chemicals act together in defining the overall selective conditions for the microbiome in the environment under investigation. For example, it is important to have an understanding of how various pharmaceuticals can have synergistic effects, and how heavy metals and other compounds (e.g., pesticides, other pharmaceuticals) provide mixtures of chemicals with possibly elevated effects in the

environment. A broad range of pharmaceuticals other than anti-infectives have antimicrobial effects (see Lagadinou et al 2020, and references within). Levels of antimicrobials below the minimum inhibitory concentration (MIC) are also recognized to have selective properties. The ecological footprints of commonly used pharmaceuticals should therefore be reviewed as possible co-selectors for AMR development. However, the standardization of methods to determine the effect of sub-MIC levels within the broader field of mixture toxicology has yet to be established in an international regulatory context.

The lack of standardized approaches means that environmental studies often vary in experimental approaches, thereby limiting the opportunity for comparative analyses such that most studies of environmental populations of bacteria cannot be compared directly with the results from other studies. Moreover, many studies do not attempt to investigate resistance dynamics over time, and rarely describe concentrations of resistance drivers in the sampled locations. It is possible that no relevant selection for AMR may be found in the environment under investigation, and thus determination of concentration ranges would not be relevant. In such cases, such experimental studies could benefit from employing more explicit hypotheses regarding how the observed resistance traits have entered the sampled populations (e.g., via migratory animals, including birds; human travel, severe weather conditions, etc.).

The Panel is of the opinion that some progress has been made in our understanding of AMR in nature and the environment since 2014 (FHI, 2014). The suggestions and conclusions of the FHI report remain valid. Although more results have been published, the majority of these are from descriptive, point-prevalence surveys that provide a snapshot of the resistance situation in a sampled bacterial population at a limited study site. Frequently, only a restricted set of known resistance traits are considered, time aspects are rarely considered, and the presence of selective agents in the same environment is usually not investigated.

Although descriptive, point-prevalence investigations may provide useful information in clinical settings, where antimicrobial therapy is routinely administered according to guidelines and treatment regimens could be adjusted according to the results obtained, such studies are of less value when investigating natural populations of bacteria at a single time point, and that have not been intentionally exposed to antimicrobials, or the levels to which they have been exposed are unclear. Such data collected on AMR at a given site will rapidly be "out of date", and will lack contextualization that could have been obtained by longitudinal sampling of the same populations (as often done in clinical settings).

In the wildlife report (2018), migratory birds were identified as a potentially important mechanism for rapid dispersal of resistance traits across continents. Moreover, anthropophilic prey species, such as mice and rats, were suggested to form a bridge between human/farm habitats, wildlife, and migratory dispersal routes. This mechanism could be further explored with the aim of understanding its significance and to identify indicator bacteria that can be monitored at the international level.

For a given environmental AMR trait, it is currently not possible to define whether that trait was naturally present prior to introduction of pharmaceutical antimicrobials, whether it emerged due to directional selection, whether its occurrence reflects the global spread of mobile genetic elements (transposons/plasmids), or whether it is due to chance distribution and the occurrence of particular microbes in the specific study site. The presence of a trait in a given environment can indicate a combination of such mechanisms, and this can differ

between sites, traits, and time. Such dynamics are rarely explicitly considered in environmental AMR studies.

However, the identification of identical resistance traits (and combinations thereof – preserved synteny) suggest recent origin and dispersal through HGT mechanisms. Directionality may remain challenging to prove, highlighting the need for a One Health approach and the importance of determining which data should be collected such that directionality, dynamics, and the key pathways and sites of gene transfer can be established.

Another limitation related to most point-prevalence studies, is the focus tends to be targeted on some particular resistance genes that are usually defined through the specific PCR used to amplify DNA extracted from the sampled bacteria. Studies of the whole microbiome of a given environment are still rare, due to the cost and practical limitations associated with immensely large numbers of bacteria/genomes. Nevertheless, only investigating particular populations (limited sample size) and genes (limited PCR targets) severely compromises the resolution and diversity of resistance detected. Moreover, for both PCR and metagenomics-based approaches, expression characteristics and the potential for HGT may be overlooked.

Major technological progress has been made in metagenomic opportunities, providing the possibility to obtain information on the full genome content of an environmental sample, as well as single whole genome sequencing (WGS). Although clearly with the potential to contribute new knowledge, the technique is not yet commonplace, and challenges in relating identified genotypes to phenotypes are still extensive, such as determining the extent to which an identified resistance gene is actually functional, expressed, and expressed at levels of clinical importance. Moreover, the definition of an ARG is not entirely clear-cut, and identification of such genes usually requires knowledge of the gene's properties. In many cases, the horizontal mobility of an ARG will determine its possible impact, and this cannot necessarily be easily inferred from sequence data. In some cases, mobility will depend on the recipient host cell, illustrating the importance of understanding context in interpreting such data.

Building coordinated international efforts, including research that goes beyond descriptive approaches, standardizing sampling and analysis regimes, promoting data accessibility on the use and selective interactions of selective agents, and developing monitoring that includes longitudinal sampling would all contribute towards filling current data gaps.

**Summary of uncertainties and data gaps regarding AMR in nature and the environment in 2020.**

The 2015-2020 reports considered here all describe various challenges in identifying scientific studies relevant to the various Terms of Reference. In addition, sources of uncertainties and numerous data gaps are described from articles considering environmental populations of bacteria. These include large population sizes, extensive (undescribed) diversity, unknown population dynamics, lack of information on migration and dispersal patterns and mechanisms, unclear relationships between contingencies, chance, and deterministic processes, absence of timelines for observing relevant population/genetic changes, variations in anthropogenic activities at the study sites, and variable properties, including dispersal and decay rates, of selective agents.

Current challenges and sources of uncertainty include:

Sampling limitations severely limit our insights into the prevalence of ARB and ARG in the environment. This is due to the high densities of bacterial populations and their native, as well as acquired, ARG. These rapidly saturate sampling efforts, so most studies describe only a few sites with a low number of samples. Alternatively, studies are limited to a particular set of common (indicator) bacterial species (as for studies in clinical settings). In this context, indicator bacteria can be defined as commonly occurring species, with known ecology and population structure, and that can be easily sampled/cultured and hence followed over time. Limiting the species diversity to a defined set of indicator bacteria enables broader screening, but the distribution of AMR in a given environment may not be revealed.

This difference in the core aim between environmental studies and most clinical studies is relevant. Most clinical studies describe the resistance characteristics/dynamics of a well-known bacterial pathogen, that has usually been grown/enriched on selective media. In contrast, environmental studies often investigate complex, not fully described, heterogeneous microbial communities, with the aim of describing the overall AMR situation in that particular environment. Given current methodological constraints, such ambitions are difficult to achieve. It is therefore important that developing appropriate methodology remains an important goal of environmental research on AMR. Two approaches are currently relevant: 1. further adopting clinical approaches that focus on a narrow set of indicator bacteria, and 2. developing tailored methodology that enables robust descriptions of the diversity of AMR in heterogeneous bacterial populations, including functional characterization.

Determining the concentrations of selective agents in the environment also presents similar challenges. A major problem is efficient extraction and sensitive analysis from heterogenous samples. Moreover, analytical techniques must be developed on a per-analyte basis; this is quite different from, for example, PCR-based analyses of genes or bacteria, where a protocol can be modified to target a broad range of known ARG by implementing some minor adjustments. Another complicating factor for investigating concentrations of selective agents in the environment is that antimicrobials have varying decay rates that are not fully understood.

Post-therapy excretion of antimicrobials in human and animal faeces is likely to contribute to the level of AMR found in community and environmental pathogens and commensals. This phenomenon, known as the **eco-shadow concept**, can be used as a basis for investigating the environmental impacts of antimicrobials (Midtvedt, 2001) and could receive more emphasis. Different substances will "cast different shadows" according to their stability, distribution, and decay characteristics.

Given the limitations outlined above, most studies available for developing risk assessments are rarely quantitative in nature, and the point-prevalence descriptions published do not facilitate broader understanding of the AMR situation in the bacterial population described, not at any other level. Indeed, prevalence-based studies rarely provide a description of the relevant larger microbial population, and simply supply a "snapshot", usually of a poorly defined larger population.

Another source of uncertainty concerns the relevant timescales for the processes involved in development of AMR. The timeframe relevant for possible changes in the resistance levels of environmental microbes is far from clear. Clinical surveys are published annually in some countries, including Norway, but how often environmental surveys should be conducted to

capture changes in AMR levels has not yet been determined, nor the response time of such communities, considered in a One Health context.

A conceptual challenge is the lack of a defined theoretical framework that can guide further experimental studies, beyond being mainly descriptive. Demonstration of causality and main pathways for resistance flow in an ecological system remains a goal. Currently, some level of clustered dispersal of resistance is expected, although approaches that explicit acknowledge such patterns remains to be developed on a broad scale. In the absence of a robust framework to map gene flow we are unable to explain the evolutionary pathways that have led to our current resistance situation.

Another key conceptual challenge and major source of uncertainty is the unknown relationship between antimicrobial usage, current levels of AMR, and future resistance patterns that will evolve. AMR development will follow different paths in different locations and different situations, and a straightforward linear relationship is unlikely in most instances. Factors of importance, beyond classic microbiology, could include migration, chance encounters (which could be modelled), national policies, changes in demand etc. A quantitative understanding of exposure versus system response should be developed, but will vary according to situation. Without such insights, the following statement remains valid: "At present, it is not clear to what extent environmental antimicrobial resistant bacteria and AMR genes promote the acquisition and spread of AMR among clinically relevant bacteria, or whether AMR genes that are acquired by both clinically relevant bacteria and strictly environmental bacteria originate from the same reservoirs"; Berendonk et al. (2015).

It is important to emphasis that AMR in the environment only becomes an issue of clinical importance, if and when humans or animals interact with the environment in such a manner that resistance traits or pathogenic bacteria are transferred (back) into these hosts.

**Some sources of knowledge gaps include:**

- A lack of harmonized sampling and analysis methods and protocols mean comparing the results from different studies or in different systems remains challenging.
- A lack of understanding of the genetic interactions and spread that occur in environmental bacteria.
- Insufficient knowledge on the ecological roles of antimicrobial agents in the natural environment, in particular the effects at sub-inhibitory levels.
- Limited knowledge on mixture toxicology/cocktail effects of antimicrobial agents (antibacterial, antifungal, PTM, disinfectant agents) for development of AMR in environmental bacteria.
- Limited quantitative/population-scale data of antimicrobial residues (antibacterial agents, antifungal agents, disinfectant agents, PTM, and other substances), ARB, and ARG in environments to which human/animals are exposed.
- Insufficient understanding of the spatial and temporal dynamics of antimicrobial agents, ARG, and ARBs.

- Limited knowledge regarding the abilities of WWT technologies (physical, chemical, or biological) to reduce residues of antimicrobial agents and their metabolites, ARB, and ARG.
- Limited quantitative data on the occurrence of PTM resistance in bacteria in WW.
- Limited knowledge on the occurrence of disinfectant resistance in bacteria in WW.
- Limited knowledge on the occurrence of antifungal agents in WW.
- A lack of understanding of spatial and temporal resistance epidemiology, including the possible role of bridging species.

# 5-Answers to the TOR

**Assignment from NEA:**

1- The NEA requested VKM to compile a report on the status and challenges from relevant VKM reports prepared during the period 2015-2020, cf. Appendix.

See the following sections 3-1, 3-2, 3-3, 3-4, 3-5, 3-6, and 3-7.

2- The NEA requested that, based on VKM reports on the topic of AMR in the period 2015-2020, VKM compiles a brief summary of how VKM perceives the significant challenges in the field of AMR in nature and the environment in 2020. The assignment includes ARB, ARG, and resistance drivers.

See section 4

# 6-Recommendations

The panel recommends that emphasis on the following areas could contribute towards a better understanding of AMR in the environment and assist in closing the knowledge gaps and decreasing uncertainty in risk assessments on this topic:

1. **Standardization of methods.** Internationally standardized methods are needed for comparative analyses and time series.
2. **Definition of bioindicators.** An internationally agreed set of bioindicators (bacterial species, genes/vectors, selective agents) are needed to focus efforts and recognize the enormous diversity of possible analytes.
3. **Development of a methods framework for cataloguing AMR development in the environment.** Current research on environmental AMR is often driven by methods that have been developed for use in clinical settings. It is necessary that

environmental AMR research develops tools that are appropriate for use in environmental studies. For instance, tools to quantify resistance changes in the environment are required, and extend beyond observing dispersal of clinical isolates.

4. **Data-expiration challenges.** Approaches that go beyond point-prevalence studies of resistance should be developed; observations from studies that cannot be directly repeated rapidly expire in terms of current resistance levels.
5. **Time-series analyses**. Increased focus is needed on the dynamics of species and ARG prevalence to enable significant changes to be recorded.
6. **Understanding relevant timescales**. Theoretical frameworks are needed to understand the relevant timescales in which changes may occur in a given environment.
7. **Decay rates**. In cases where dispersal is driving high levels of AMR in natural environments, absence of exposure should reduce levels. More knowledge of such dynamics, and the different mechanisms behind them, is needed.
8. **Resistance complexity**. Increased focus on disentangling the interplay between host dispersal, vector mobility, and amplification/selection in determining observed resistance levels is needed, along with acknowledgement of the complexity of these interactions.
9. **(Multi)directionality**. Increased focus is needed on bioindicators and approaches that can be meaningful in establishing the directionality of gene flow between environments. This should take into account that the magnitude of effects is not necessarily due to the size of the exposure alone.
10. **Mixture toxicology.** Standardized methods to describe the effects of selective agents acting together in mixtures are necessary.

### Bridging species

In the wildlife report (Nielsen et al., 2018), migratory birds were identified as a possible important mechanism for rapid dispersal of resistance traits across continents. Moreover, anthropophilic prey species, such as mice and rats, were proposed to form a bridge between human/farm habitats, wildlife, and migratory dispersal routes. We recommend that the role of human-wildlife interfaces, bridging species, and their possible importance as dispersal pathways are further explored with the aim of elucidating their significance, and also to identify relevant indicator bacteria that can be monitored. This would contribute to our understanding of the spatio-temporal epidemiology of AMR in different environments.

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## Appendix I

1- Assessment of antimicrobial resistance in the food chains in Norway
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2- Assessment of the transfer of antimicrobial resistance between pets and humans in Norway
https://www.vkm.no/risikovurderinger/allevurderinger/vurderingavoverforingavbakteriermedantimikrobiellresistensmellomkjaledyrogmennesker.4.2994e95b15cc545071613c50.html

3- The risk of development of antimicrobial resistance with the use of coccidiostats in poultry diets

https://www.vkm.no/risikovurderinger/allevurderinger/risikovurderingavutviklingavantimikrobiellresistensvedbrukavkoksidiostatikaifjorfefor.4.2994e95b15cc545071615293.html

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6- Antimicrobial resistance in wildlife - potential for dissemination (2018)
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