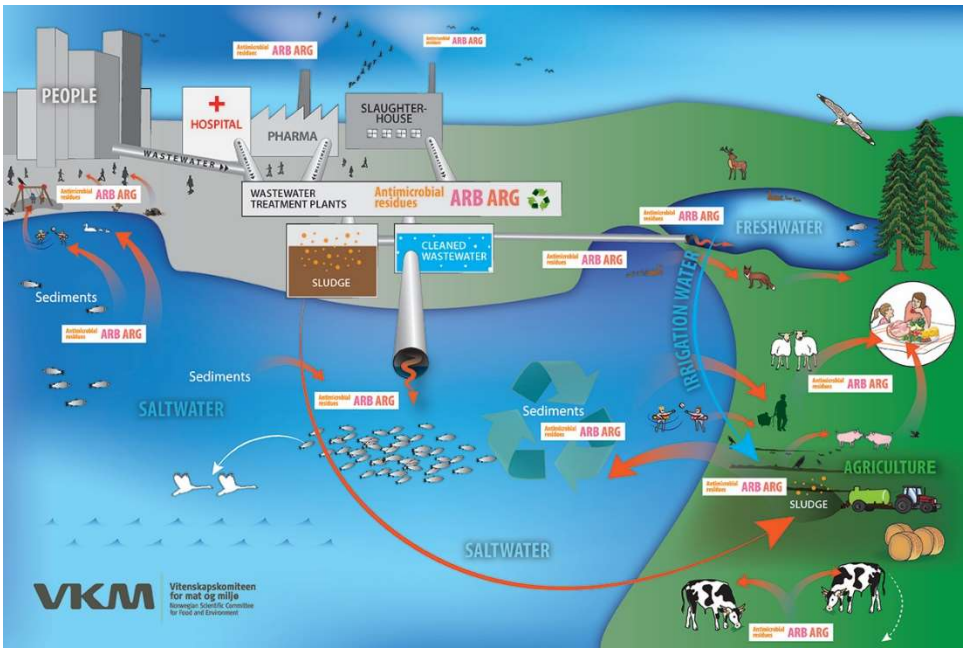
Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment



VKM Report 2020: 08

Assessment of the impact of wastewater and sewage sludge treatment methods on antimicrobial resistance

Scientific opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food and Environment

Report from the Norwegian Scientific Committee for Food and Environment (VKM) 2020: 08

Assessment of the impact of wastewater and sewage sludge treatment methods on antimicrobial resistance

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Assessment of the impact of wastewater and sewage sludge treatment methods on antimicrobial resistance

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 Food Safety Authority. The project group consisted of eight persons, and a project leader from the VKM secretariat. Two external referees reviewed and commented on the manuscript. The VKM Panel on Microbial Ecology evaluated and approved the final opinion drafted by the project group.

Authors of the opinion

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Parts of the text in chapter 4.4 are proposed by the Norwegian Environment Agency.

Members of the VKM Panel on Microbial Ecology that assessed and approved the opinion: In addition to Yngvild Wasteson (chair of the Panel), Erik Joner and Elisabeth Henie Madslien, these were (in alphabetical order):

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Acknowledgment

VKM would like to thank the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food and Environment for comments and views on this opinion. VKM acknowledge the referees Arnfinn Sundsfjord (The Arctic University of Norway, Department of Medical Biology) and Øyvind Ørmen (Norwegian University of Life Sciences, Department of Food Safety and Infection Biology) for valuable reviewing and commenting on the manuscript. The project leader from the VKM secretariat, Tron Øystein Gifstad, is acknowledged for coordinating the work with the opinion.

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.

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Key words: VKM, Norwegian Scientific Committee for Food and Environment, Norwegian Environment Agency, wastewater, wastewater treatment, effluent water, sludge, antimicrobial agents, antimicrobial resistant bacteria, antimicrobial resistance genes

Summary

The request from NFSA and NEA:

Antimicrobial agents and microorganisms are introduced to sewage systems by different human activities, from private homes, institutions such as schools and hospitals, office buildings, industrial and commercial activities, i.e., from everywhere where people work and live.

The Norwegian Food Safety Authority (NFSA) and Norwegian Environment Agency (NEA) asked the Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) for an extension of the 2009 VKM report "Risk assessment of contaminants in sewage sludge applied on Norwegian soils" regarding the impact of wastewater (WW)- and sewage sludge treatment methods used in Norway, on the fate and survival of antimicrobial resistant bacteria, fate of antimicrobial resistance genes, and main drivers for resistance (e.g. antibiotics, antifungal agents, heavy metals, disinfectants).

The request addressed by VKM:

VKM appointed a working group, consisting of three members of the Panel on Microbial Ecology, four external members and VKM staff to prepare a draft Opinion document. The Panel on Microbial Ecology has reviewed and revised the draft prepared by the working group and approved the Opinion document "Assessment of the impact of wastewater and sewage sludge treatment methods on antimicrobial resistance".

The antimicrobial resistance cycle:

Exposure to antimicrobial agents is regarded as the most important driver for development and dissemination of AMR in microorganisms. Consequently, an important location for the development of AMR is the gut of humans or animals receiving antimicrobial drug therapy. As ARB, ARG, resistance genes and antimicrobial agents will end up in the WW system, this system could be regarded as a potential hot spot for interactions between different microorganisms, between different antimicrobial agents, and between microorganisms and antimicrobial agents. Hospitals and pharmaceutical companies are regarded as being an important source for antimicrobial drug residues released in WW.

At the wastewater treatment plant (WWTP), bacteria and genes end up either in the effluent wastewater fraction or in the sludge fraction. When ARB and ARG are distributed with the WW sludge, they may reach arable land when the sludge is used as soil improver and fertilising product, and thus be recycled into the food-production chain. When following the effluent WW fraction, ARB and ARB will be released into WW recipients, such as lakes, rivers or fjords, and may, from these environments, also be recycled into food production. In each step of these cycles, ARB and ARG will be introduced into new environmental compartments to which they must adapt, and to microbial communities with which they must compete for

survival and growth. Depending on the bacterial species, these new environmental compartments will be more or less hostile, but they will also provide opportunities for microbial interactions, like dissemination of ARG due to horizontal gene transfer (HGT) within and between bacterial species.

Findings:

It is challenging to deliver a general assessment of the nature of as well as the probability for direct discharge of ARB and ARG into effluent WW and applied sludge. This is due to the combined complexity of resistance carriers, traits, various sources of variation, and the WW systems. Moreover, there is currently a lack of harmonized methods and protocols to compare studies from different systems. However, there are no strong indications that there is a significant enrichment of ARB in WWTP operated under European conditions, which, on a general level, also applies to the Norwegian situation. Although some studies indicate a slight increase in the fraction of ARB, the absolute reduction in bacterial load during WW treatment (WWT) is significant; removal of between 99 % to 99.9 % of faecal indicator bacteria is generally achieved by secondary treatment, including biological and physico-chemical treatment steps.

Effluent WW is often released into water recipients, and there are many mechanisms (physical, mechanical, and chemical) that will limit the extent that ARB of faecal origin are transferred to the food-production chain. However, there are different views on the significance of this release for the development of AMR. Results from single studies indicate that WWTP effluents contribute little to the total AMR exposure of micro – and macro organisms in aquatic and marine environments. On the other hand, freshwater environments in general are regarded as an important reservoir of novel antibiotic resistance determinants, and in some areas, relative abundance of resistance determinants in effluents has been observed to be considerably higher than in pristine natural water sources. Some imprint of AMR in recipient waters, compared to pristine waters, is unavoidable.

During WWT, bacteria largely adhere to particles that are aggregated and precipitated to form a solid sludge. The mandatory hygienisation of sludge kills a large proportion of these bacteria, notably all thermosensitive faecal bacteria. However, the resulting hygienised sludge is still rich in bacteria, some of which are carriers of ARGs. The current Norwegian regulations on use of sludge on soil contribute to prevent contamination of food with antimicrobial resistant bacteria and antimicrobial resistance genes from sludge. Yet, soils do contain a pool of both natural and sludge-derived antimicrobial resistance. The contribution of sludge to this antimicrobial resistance pool is probably temporally limited to a period after soil amendment with sludge. A recent, comprehensive study from Sweden showed that long-term application of sewage sludge on farmland only resulted in minor changes of soil bacterial community composition. No evidence could be found for enrichment of antimicrobial resistant bacteria or antimicrobial resistance genes in soil amended with digested and stored sewage.

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 effluent water from the WWTP, and this might suggest that local treatment of the WW at the hospital could be advantageous. A recent Norwegian study monitored bacterial diversity in different WW in the Oslo area, and found the highest concentration of AMR (ARB and/or ARG) in hospital WW. But surprisingly, high concentrations were also found in the studied community wastewater. The relative contribution of hospital effluents seemed low in terms of dissemination of antimicrobial resistant bacteria to the wastewater treatment plant.

All measures that can be taken at source to avoid dissemination of antimicrobial agents, ARB, and ARG should be evaluated for their contribution towards combatting AMR emergence. Concentrations of antimicrobial agents, ARB, and ARG are highest in the sewage system and at the inlet to WWTPs. Separation of the different fractions of antimicrobials, ARB, and ARG for individual treatment may therefore reduce the total load reaching the WWTPs. Due to the high concentrations of ARB and ARG in the sewage system, risks from sewage pipe leakages are of concern. Intrusion of contaminated water into the drinking water distribution system should also raise concern. Rehabilitation of the sewage and drinking water networks will considerably mitigate risks. The level of sewage treatment in Norway is rather low, and upgrading will decrease the concentration of bacteria discharged. However, WWTPs are generally not designed for removal of AMR. Membrane processes seem to be the most promising option for increasing such removal rates.

Future perspectives:

The opinion discusses how the "concept of sensitive recipients" for requirements of the level of WWT could be revisited. This concept is currently based on controlling nutrient loads to the environment, rather than on trace contaminants or contaminants such as ARB and ARG that develop in the stressed environment. In the future, it might be of value to define requirements for WWT based on the relative increase caused by the discharge to the pollution level. Using such a paradigm, a small load with contaminants to a rather unpolluted environment would be rated as being highly critical and the discharge would require further treatment. In addition to the amount of ARB, the type of resistance and their level of horizontal mobility are also important in this aspect.

This opinion also proposes the establishment of a new monitoring programme, parallel to the existing NORM and NORM-VET monitoring programmes; "NORM-ECO". There is relatively little knowledge on AMR in non-clinical compartments, compared with hospital and other clinical settings, and parameters that would trigger immediate responses from NFAS or NEA are not yet identified. However, establishment of a "NORM-ECO"-system requires clarification of that needs further definition.

Sammendrag på norsk

Oppdrag fra Mattilsynet og Miljødirektoratet:

Antimikrobielle stoffer og mikroorganismer skilles ut til avløpssystemene gjennom ulike menneskelige aktiviteter; fra private hjem, forskjellige institusjoner inkludert skoler og sykehus, kontorbygg, industriell og kommersiell virksomhet, dvs. overalt hvor mennesker bor og lever.

Mattilsynet og Miljødirektoratet ba Vitenskapskomiteen for mat og miljø (VKM) om en utvidelse av VKM-rapporten fra 2009, «Risikovurdering av avløpsslam som jordforbedringsmiddel», angående effekt av behandlingsmetoder for avløpsvann- og slam brukt i Norge på utvikling av bakterier som er resistente overfor antimikrobielle stoffer, spredning av antimikrobielle resistensgener, og drivere for utvikling av resistens (som antibiotika, soppdrepende midler, tungmetaller, desinfeksjonsmiddel, osv.).

Slik ble oppdraget utført av VKM:

VKM oppnevnte en arbeidsgruppe bestående av tre medlemmer fra faggruppen for mikrobiell økologi, fire eksterne medlemmer og VKM-ansatte, for å svare på spørsmålene i oppdraget. Faggruppen for mikrobiell økologi har gjennomgått og revidert rapportutkastet fra arbeidsgruppen og godkjent rapporten.

Den antimikrobielle resistenssyklusen:

Eksponering for antimikrobielle stoffer blir sett på som den viktigste pådriveren for utvikling og spredning av antimikrobiell resistens hos mikroorganismer. Tarmen hos mennesker eller dyr som får antimikrobielle medikamenter, er dermed et viktig sted for utvikling av antimikrobiell resistens. Ettersom resistente bakterier, resistensgener og antimikrobielle stoffer skilles ut til avløpssystemet, kan avløpssystemet være en potensiell «hot spot» for interaksjoner mellom forskjellige mikroorganismer, mellom forskjellige antimikrobielle stoffer, og mellom mikroorganismer og antimikrobielle stoffer. Det er særlig risiko for at sykehus og farmasøytisk industri kan tilføre antimikrobielle medikamenter og medikamentrester til avløpsvannet.

Fra renseanlegg for avløpsvann vil bakterier og gener havne enten i avløpsvann eller i slam som slippes ut fra anlegget. Når slam brukes som jordforbedringsmiddel og gjødsel, kan resistente bakterier og resistensgener nå dyrkbar jord og dermed resirkuleres til matproduksjonskjedene. Via renset avløpsvann blir resistente bakterier og resistensgener frigjort til resipienter, det vil si elver, innsjøer eller fjorder, og kan resirkuleres til matproduksjonen fra disse miljøene også. I hvert trinn i syklusene som er beskrevet, vil resistente bakterier og resistensgener bli introdusert til nye miljøer som de må tilpasse seg, og til mikrobielle samfunn hvor de må konkurrere om næring for å formere seg og overleve. Avhengig av bakterieart vil disse nye miljøene være mer eller mindre uvennlige, men

miljøene vil også gi muligheter for mikrobielle interaksjoner, som spredning av resistensgener, på grunn av horisontal overføring i og mellom bakteriearter.

Viktige funn beskrevet i rapporten:

Det er vanskelig å foreta en samlet og generell vurdering av sannsynligheten for direkte utslipp av resistente bakterier og resistensgener i avløpsvann og slam, på grunn av kompleksiteten i avløpssystemer og mangelen på harmoniserte metoder og protokoller for å sammenligne data fra forskjellige systemer. Det er imidlertid ingen sterk indikasjon på at det er en betydelig seleksjon av resistente bakterier i renseanlegg for avløpsvann under europeiske forhold (for eksempel endemisk nivå av resistensgener, bruk av teknologi, liten produksjon av antibiotika), noe som generelt også gjelder for situasjonen i Norge. Selv om resultater fra noen enkeltstudier indikerer at behandlingsprosessene kan gi en liten økning i andelen resistente bakterier, er den absolutte reduksjonen gjennom behandlingen betydelig: mellom 99 % og 99,9 % av fekale indikatorbakterier (Mellom 99 % og 99,9 % av fekale indikatorbakterier, dvs. bakterier som stammer fra avføring, vil fjernes gjennom osv.) vil fjernes gjennom en sekundær behandlingsprosedyre, som inkluderer biologiske og fysisk-kjemiske behandlingstrinn.

Avløpsvann slippes ut i elver, innsjøer og fjorder, og det er mange fysiske, mekaniske og kjemiske mekanismer som begrenser sannsynligheten for at resistente bakterier av fekal opprinnelse blir tilbakeført til matproduksjonskjedene. Imidlertid er det ulike synspunkter på hvilken betydning slike utslipp har for videre utvikling av antimikrobiell resistens. Resultater fra enkeltstudier indikerer at renset avløpsvann bidrar relativt lite til den totale resistenseksponeringen som organismer i vannmiljøer og marine miljøer utsettes for. På den annen side blir ferskvannsmiljøer generelt sett på som et viktig reservoar for nye resistensdeterminanter, og i noen områder har forekomsten av resistensdeterminanter i renset avløpsvann blitt observert å være betydelig høyere enn i uberørte naturlige vannkilder. Det ser ut til å være uunngåelig med et visst omfang av antimikrobiell resistens i resipienter sammenlignet med uberørte vannkilder.

Under behandlingsprosessene i renseanleggene fester bakterier seg i stor grad til partikler som aggregerer og deretter felles ut for å danne et fast slam. Ved å fjerne smitte- og giftstoffer fra slammet forskriftsmessig, drepes en stor andel av disse bakteriene, særlig fekale bakterier som er følsomme for høye temperaturer. Imidlertid er det hygieniserte slammet fortsatt rikt på bakterier, og noen av disse er bærere av spesifikke resistensgener. Det norske regelverket om bruk av slam på jord, vil imidlertid bidra til å begrense at mat forurenses med resistente bakterier og resistensgener. Jordsmonn har uansett et variert innhold av resistente bakterier. Mange er naturlig tilstede i jord og noen kan være tilført med slam. Den relativt sett største betydningen av slambakterier er sannsynligvis begrenset til en kortvarig periode etter at slammet er pløyd ned i jorda. En ny og omfattende studie fra Sverige viste at anvendelse av avløpsslam på jordbruksmark over flere år kun resulterte i mindre endringer i populasjonen av jordbakterier. Det ble ikke funnet noen bevis for at bruk

av behandlet avløpsslam og noen økning i mengden av resistente bakterier eller resistensgener i jorda.

Avløpsvann fra sykehus har gjerne et høyere innhold av resistente bakterier, resistensgener og rester av antimikrobielle stoffer enn avløpsvann fra husholdninger og samfunnet for øvrig. Dersom avløpsvann fra sykehus går til store renseanlegg, betyr det ikke nødvendigvis at renseanlegget vil slippe ut større mengder resistente bakterier eller resistensgener. Derimot kan utslipp fra et sykehus til et mindre renseanlegg ha større innvirkning totalt sett, noe som betyr at man bør vurdere å behandle avløpsvannet ved sykehuset før det slippes ut på det ordinære avløpsnettet. Nylig publiserte resultater fra en norsk studie som undersøkte bakteriepopulasjonene i forskjellige avløpsvann i Oslo-området, viste at de høyeste konsentrasjonene av antimikrobiell resistens ble funnet i urenset avløpsvann fra sykehus. Det ble også funnet overraskende høye konsentrasjoner i urenset avløpsvann fra husholdninger. Det relative bidraget fra sykehusavløpet var av mindre betydning når det gjaldt tilførsel av resistente bakterier til renseanlegget.

Antimikrobiell resistens er beskrevet som en av vår tids største folkehelsetrusler. For å bekjempe trusselen, må alle tiltak for å unngå spredning av antimikrobielle stoffer, resistente bakterier og resistensgener vurderes. Konsentrasjonen av antimikrobielle stoffer, resistente bakterier og resistensgener er høyest i ubehandlet kloakk og ved innløpet til renseanleggene. Å separere de forskjellige bestanddelene som har høye konsentrasjoner av antimikrobielle stoffer, resistente bakterier og resistensgener og for å behandle dem separat, kan derfor være et effektivt tiltak for å redusere den totale belastningen som kommer til renseanleggene. På grunn av de høye konsentrasjonene i urenset avløpsvann, er det viktig å være oppmerksom på risikoen for lekkasjer fra rørsystemene for slikt avløpsvann, spesielt når det gjelder risiko for inntrenging av avløpsvann til distribusjonssystemet for drikkevann. Oppgradering og vedlikehold av avløps- og drikkevannsnettverk vil redusere denne risikoen betydelig. Metodene som brukes for rensing av avløpsvann i Norge er generelt relativt enkle. En oppgradering til mer avanserte metoder vil redusere konsentrasjonen av alle bakterier som frigjøres fra anleggene. Imidlertid er ikke renseanlegg for avløpsvann i utgangspunktet designet for å fjerne antimikrobiell resistens. Membranbaserte metoder ser ut til å være de mest lovende alternativene for å forbedre et slikt rensetrinn.

Fremtidsperspektiver:

I rapporten drøftes hvordan konseptet med «sensitive resipienter» kan inkluderes når det gjelder revisjon av krav til rensing av avløpsvann. I dag er dette konseptet basert på vurdering av tilførsel av næringsstoffer til miljøet, snarere enn på sporforurensninger eller forurensninger som resistente bakterier og resistensgener som utvikler seg i et utsatt miljø. Det kan være fornuftig å definere krav til rensing av avløpsvann i relasjon til den effekten en økning av utslipp vil medføre. Ved å bruke en slik tilnærming, vil en liten forurensingsbelastning til et i utgangspunktet rent miljø bli vurdert som svært kritisk, og utløse krav om ytterligere behandling. I tillegg til å vurdere mengde av forurensing, vil det i

dette perspektivet også være nødvendig å vurdere type forurensning. Det vil si type resistens og eventuell lokalisering av resistensdeterminanter på kjente, mobile elementer.

I rapporten drøftes også etablering av et nytt overvåkningsprogram som kan gå parallelt med de eksisterende overvåkningsprogrammene NORM og NORM-VET - «NORM-ECO». Det er fremdeles relativt liten kunnskap om antimikrobiell resistens i ikke-kliniske miljøer, og det er ikke identifisert noen målbare parametere som vil utløse umiddelbar respons fra Mattilsynet eller Miljødirektoratet. Imidlertid krever etablering av et «NORM-ECO» -system avklaring av en rekke spørsmål, som må besvares gjennom ny forskningsinnsats.

Abbreviations and/or glossary

Abbreviations

AMR Antimicrobial Resistance

ARB Antimicrobial resistant bacteria

ARG Antimicrobial resistance genes

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

BOD Biological oxygen demand

COD Chemical oxygen demand

CPE Carbapenemase producing *Enterobacteriacae*

DAA Dekar

DAEC Diffusely adherent *Escherichia coli*

DDD Defined daily dose

DW Dry weight

EAEC Enteroaggregative *Escherichia coli*

ECDC European Centre for Disease Prevention and Control

EIEC Enteroinvasiv *Escherichia coli*

EPA Statistics Norway and Norwegian

EPEC Enteropatogen *Escherichia coli*

ESBL Extended Spectrum Beta Lactamase

ESBL-E ESBL-producing *Enterobacteriaceae*

ESBL-EC ESBL-producing *E. coli*

ESKAPE *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species

ETEC Enterotoxic *E. coli*

EU European Union

EXPEC Extraintestinal pathogenic *Escherichia coli*

GAC Granular activated carbon

HGT Horizontal gene transfer

MAR Multiple antibiotic resistance

MBR Membrane Bioreactor

MCC Minimum metal co-selective concentration

MDR Multidrug resistant Multidrug resistance

MGE Mobile genetic element

MIC Minimum inhibitory concentration

MRSA Methicillin-resistant *Staphylococcus aureus*

MSC Minimum selective concentrations OR microbial selection concentrations

MWCO Molecular weight cut off

NEA Norwegian Environment Agency

NFSA Norwegian Food Safety Authority

NORM The Norwegian monitoring programme for AMR in human pathogens

NORM-VET The Norwegian monitoring programme for AMR in animal pathogens

OTC Over-the-counter

PAC Powdered activated carbon

PBP Penicillin-binding protein

PCR Polymerase chain reaction

PE Person equivalents

PTM Potentially toxic metals

PU Person units

QACs Quaternary ammonium compounds

QRDR Mutations in the quinolone-resistance determining region

RD Resistance drivers

ROS Reactive oxygen species

SS solid substances

ST Sequence type

ToR Terms of reference

UTI Urinary tract infection

UWWT Urban Wastewater Treatment Plant

VKM Norwegian Scientific Committee for Food and Environment

VRE Vancomycin-resistant Enterococci

VRSA Vancomycin-resistant *Staphylococcus aureus*

WHO World Health Organization

WHOPPL WHO priority pathogens list

WW Wastewater

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.

Antibiotics: 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.

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

Bactericidal effect: The agent kills the bacteria.

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 typically surrounded by an extracellular polymeric slime matrix. Floccs are suspended aggregates of microorganisms surrounded by an extracellular polymeric slime matrix that is formed in liquid suspension.

BOD_5: Biological oxygen demand – amount of oxygen consumed per litre of sample during 5 days of incubation at 20C

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, for example by being located together on a mobile genetic element (e.g., a plasmid, transposon, or integrin or on the chromosome).

crAssphage: Cross-assembly phage is a _ (virus that infects bacteria) that was discovered in 2014 by computational analysis of publicly accessible scientific data on human faecal metagenomes.

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

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

Effluent wastewater: The major aim of wastewater treatment is to remove as much of the suspended solids as possible before the remaining water, called effluent, is discharged back to the environment.

Fertilising product: A fertiliser, substance, mixture, microorganism, or any other material, applied or intended to be applied, either on its own or mixed with other material, to soil, plants or their rhizosphere for the purpose of imroving soil and/or providing plants with nutrients or improving their nutritional efficiency.

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 assembly platforms - DNA elements that acquire open reading frames embedded in exogenous gene cassettes and convert them to functional genes by allowing expression through a shared promoter.

Mesophilic digester: Mesophilic biodigester is a kind of biodigester that operates in temperatures between 20 °C and about 40°, typically 37 °C.

Microbiota: Collective term for microbial community (i.e., any type 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.

Resistome: The collection of genes that could contribute to a phenotype of antimicrobial resistance.

Sanitizer: A chemical agent that reduces microbiological contamination.

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 a by-product of physical (primary treatment), biological (activated sludge), and (physicochemical precipitation of suspended solids by) chemical treatment processes.

Sterilization: The process of destroying all microorganisms (including spores).

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

Thermophilic digester: Thermophilic biodigester is a kind of biodigester that operates in temperatures above 50 °C producing biogas. … In fact, it can be as much as six to ten times faster than a normal biodigester."

Transduction: 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 is capable of moving into a new position within the same or another chromosome or plasmid. Also called jumping gene.

Wastewater (WW): 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 e.g. hospitals. Often the term "wastewater" is used as a synonym for sewage. See also "Sewage".

WHO PPL: The World Health Organization was requested by Member States to develop a global priority pathogens list (global PPL) of antibiotic-resistant bacteria to help in prioritizing the research and development (R&D) of new and effective antibiotic treatments. The list was

to identify the most important resistant bacteria at a global level for which there is an urgent need for new treatments. (https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1)

Background as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

This risk assessment requested here by is a joint assignment from NFSA and NEA concerning antimicrobial resistance issues in wastewater treatment facilities, in wastewater effluent released to nature, and in sewage sludge used as fertiliser product. The NFSA and NEA considers it more effective to submit a joint request since various aspects of antimicrobial resistance relate to areas regulated by both authorities.

AMR is present in most environments and its development and spread is a worldwide concern, and is an issue, which should be considered in a one-health perspective. Studies of the presence of AMR are sporadic in different environments, but increased levels of AMR are found in environments such as soil, wastewater, treatment plants, water and sediments. The prevalence of ARBs and ARGs is expected to be higher in environments as wastewater treatment plants (WWTPs) as they serve as important reservoirs receiving wastewater from household, industry and hospitals where antibiotics are applied. Wastewater from WWTPs could constitute a source for spread of AMR into the environment, and the assessment should consider its risk.

In addition, circular economy and maximizing waste recycling is a focus of EU regulations and therefore it is important to ensure that the utilisation of sewage sludge as fertiliser product does not impair human health and the environment.

The Norwegian Governments strategy against Antibiotic resistance, 2015-2020, contains several measures to combat antibiotic resistance with focus on the development of knowledge in this field. The National Action Plan (2015-2020) to combat antibiotic resistance within the agricultural and food sector of this plan, requested the Norwegian Ministry for Agriculture and food, an updating of the 2009 VKM report "Risk assessment of contaminants in sewage sludge applied on Norwegian soils" with an assessment of the impact of sewage sludge treatment methods used in Norway, on the fate and survival of antibiotic resistance (ARB).

Terms of reference as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

The Norwegian Food Safety Authority (NFSA) and Norwegian Environment Agency (NEA) herby request the Norwegian Scientific Committee for Food and Environment (VKM) to extend the 2009 VKM report "Risk assessment of contaminants in sewage sludge applied on Norwegian soils" with issues related to antimicrobial resistance.

The desired extension of the report refers to the impact of wastewater- and sewage sludge treatment methods used in Norway, on the fate and survival of antimicrobial resistant bacteria (ARB), fate of antimicrobial resistance genes (ARG), and drivers for resistance (RD) (as antibiotics, antifungal agents, heavy metals, disinfectant agents, etc.,).

Pharmaceutical residues were assessed in general by VKM in 2009, however, the report did not thoroughly address antimicrobial resistance. VKM concluded that it is unlikely that antimicrobial resistance (AMR) may be promoted in the wastewater treatment plants (WWTP), or in the soil following application of sewage sludge as fertilising product. There is an exception for when residues of fluoroquinolones are in the sludge, as fluoroquinolones are stable in the environment, and is a potential for development of resistance.

The hereby-requested assessment should include, where possible the level ARB, ARG and RD in the wastewater effluent released to the environment, in relations to high risk and low risk sources of wastewater "donors" to the wastewater facilities. An earlier Norwegian assessment- red fox as indicator [1] showed significant differences in occurrence of resistance between medium and high population density areas.

It is also expected that the risk may vary between different wastewater treatment plants, according to wastewater sources. The assessment should be with special focus on WWTP receiving wastewater from hospitals, pharmaceuticals industry, slaughterhouses or any other sources, which are potential sources of high levels ARBs, ARGs and RDs.

An updated and extended report with the assessments requested here by, is important to gain knowledge and enable us to identify possible risk reduction measures. The risk

[1] Antimicrobial resistance in the Norwegian environment - red fox as an indicator. Norwegian Veterinary Institute Rapport 11-2017

assessment will also be utilized for evaluation of present regulations, identifying gaps in the regulations and providing guidance for the industry for achieving best practice.

With reference to above-mentioned facts, the NEA and the NFSA therefore request VKM to:

1. Describe wastewater treatment methods used in Norway today and how these methods affect the fate and survival of ARB and ARG in effluent water released to the recipient.
2. Describe the sewage sludge treatment methods used in Norway and assess the impact of these methods, on the fate and survival of ARB, ARG, and the content of RD.
3. Asses if RDs in fertilising material produced from sewage sludge play a role in the development, spreading and persistence of bacterial resistance to these elements as well as cross or co-resistance to antimicrobial agents
4. Assess possibility of treated sewage sludge posing a hazard when utilized as a fertilising material in agriculture or in green areas. Also, identify application areas where the hazard for human and animal health or the environment is expected.
5. Identify and assess various risk mitigation measures to

- reduce the probability for wastewater effluent and fertilising material containing ARB?
- reduce the probability that the wastewater effluent and fertilising materials may play a role in the development and spreading of AMR.

6. Identify indicators that can be used for monitoring and control of resistance driving chemicals (antibiotics, antifungal agents, heavy metals, disinfectant agents etc.) in wastewater effluent and sludge destined for use as fertiliser.
7. How significant is the exposure of workers, farmers and the public to AMR through production and use of sludge as a fertiliser material in Norway.
8. Evaluate the prevalence of ARB and ARG in wastewater effluent in different WWTPs with low and high exposure of potential resistance drivers (hospitals, industry, universities and household).
9. Describe the biological characteristics of the ARB and ARG identified in WWTPs

Assessment

1 Introduction

Antimicrobial agents (antibiotics, antifungals, potentially toxic metals (PTM), biocides, in particular disinfectants) are introduced into sewage systems by different human activities, such as direct disposal of residues excreted in urine and faeces, unused or expired medications, release from pharmaceutical plants and hospitals, and veterinary drug use (Christou et al., 2017). Similarly, microorganisms are introduced into wastewater (WW) systems from all types of human activities: from private homes, from institutions including schools and hospitals, office buildings, and from industrial and commercial activities. Resistant bacteria may be introduced to people via food or may develop in people due to use of antimicrobial agents. Bacteria from animals may reach the WW systems through surface run-off from faeces in the environment or through WW effluents from slaughterhouses and other food-producing enterprises.

As discussed in VKM's previous risk assessment regarding contaminants in sewage sludge applied to Norwegian soils, the most important location for development of antimicrobial resistance (AMR) is probably in the gut of humans or animals receiving antibacterial drug therapy (Sundstøl et al., 2009). Exposure to antimicrobial agents is regarded as the most important driver for development and dissemination of AMR in microorganisms. Antimicrobial-resistant bacteria (ARB) and antimicrobial-resistance genes (ARG) from the human gut are excreted into WW systems together with faeces.

The sewage system could be regarded as a potential hot spot for interactions between different microorganisms, between different antimicrobial agents, and between microorganisms and antimicrobial agents. The selection pressure for development and dissemination of AMR in sewage is exerted by dissolved antimicrobial drug residues. Hospitals and pharmaceutical companies are regarded as being at particular risk for disposing of antimicrobial drug residues into their WW.

At the wastewater treatment plant (WWTP), bacteria and genes might end up either in the effluent fraction or the sludge fraction. ARB and ARG may reach arable land when the sludge is used as soil improver and fertilising product, and could thus be recycled into the food-production chain. When following the effluent fraction, ARB and ARG will be released into recipient waterbodies, like lakes or fjords, and may, from these environments, also be recycled into food production. This is illustrated in Figure 1-1. In each step of the cycles, ARB and ARG will be introduced into new environmental compartments to which they must adapt, and to microbial communities with which they must compete for survival and growth. Depending on the bacterial species, these compartments will be more or less hostile, but will also provide opportunities for microbial interactions, like dissemination of ARG due to horizontal gene transfer (HGT) within and between bacterial species.

After application of sludge to soil as a fertilising product, selection mechanisms can occur due to antibacterial drug residue molecules in the soil compartment itself (typically in the low µg/kg soil dry weight (DW)). These molecules are transported with sludge to the topsoil and may desorb from the waste to the soil compartment. Theoretically, they can exert a selection pressure, or at least a pressure to maintain ARG in the existing soil bacteria. Probably this is a less important way of inducing AMR in the soil compartment (Sundstøl et al., 2009). It should be noted that some natural antibiotics have always been present in soil, as soil microorganisms may produce such compounds at low levels to compete with other microbes in their habitat. The soil actinobacteria genus *Streptomyces* spp. are, in fact, the original source of numerous antibiotics currently used in human medicine (D'Costa et al., 2006).

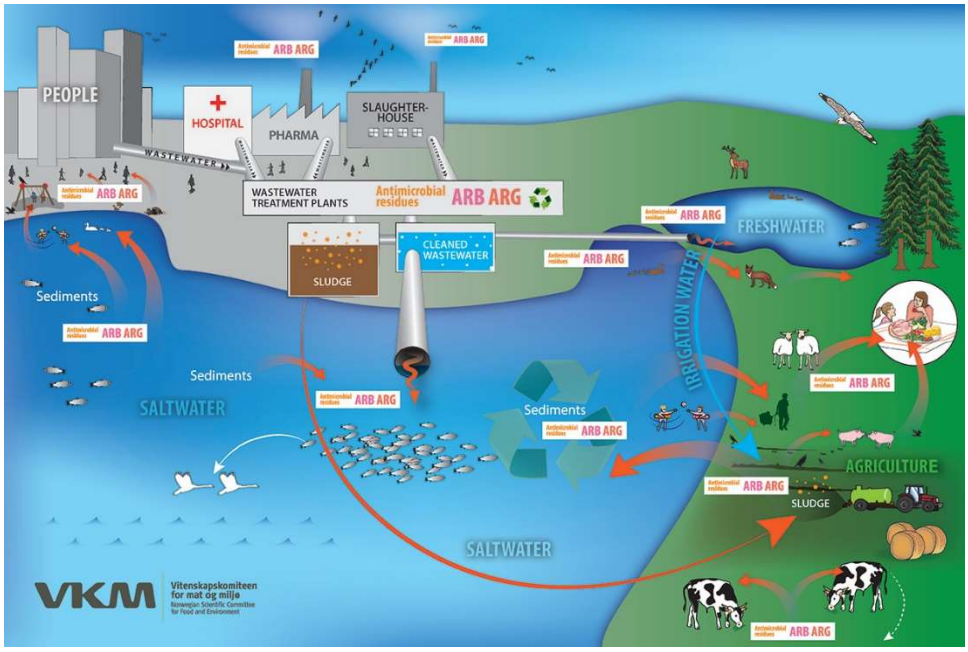

Figure 1-1. AMR in wastewater treatment processes and some possible pathways for transfer of antimicrobial residues, ARB (antimicrobial resistant bacteria), and ARG (antimicrobial resistance genes to the environment).

2 Literature and data

Literature and data used for the different topics in this opinion are as follow:

Mechanisms of action and mechanisms of resistance of antimicrobial agents (Chapter 4)

General information regarding the modes of action of antimicrobial agents, AMR, and HGT was obtained by searching using the following terms: antimicrobial resistance [Title/Abstract]) OR antibiotic resistance [Title/Abstract]) AND Review [ptyp]) in PubMed. Only the articles published in the last 10 years (2010-2019) were used in this assessment.

Other relevant information was obtained from the following reports and books:

VKM reports: AMR in the food chain (Yazdankhah et al., 2015), AMR due biocides and heavy metals (Tronsmo et al., 2016), Potentially toxic metals in soil and fertilising products (Wasteson et al., 2017), AMR in wildlife (Nielsen et al., 2018).

Goodman & Gilman's The Pharmacological Basis of Therapeutics 11th Ed., chapter 48 Antifungal Agents, ISBN 0-07-142280-3) (Brunton et al., 2019).

Pharmacology information was searched Micromedex/Martindale; IBM Micromedex® DRUGDEX®: IBM Micromedex® DRUGDEX® (electronic version). IBM Watson Health, Greenwood Village, Colorado, USA. Available at: https://www.micromedexsolutions.com/ (cited: 02/17/2020) (Micromedexsolutions, 2020).

Stability of antimicrobial agents (Chapter 4)

A search was conducted in PubMed using the terms; "antimicrobial agents" OR "antibiotics", Title/Abstract] AND "wastewater" [Title/Abstract] AND Review [Title/Abstract] using the Advanced Search Builder provided in PubMed (www.ncbi.nlm.nih.gov/pubmed) and resulted in 160 citations (March 2019). We limited our search to review articles and only PubMed and no other databases since information regarding stability of antimicrobial agents in wastewater/environment are general and several review articles not necessary represent new information. Twenty-five (25) articles fulfilled the criteria to be included in this part of this opinion.

Use of antimicrobial agents in Norway (Chapter 4)

Data regarding use of antimicrobial agents (antibiotics and antifungal agents) obtained from NORM/NORM-VET 2006, and 2018 reports (www.vetinst.no), and Drug consumption in Norway 1999-2019, Norwegian Institute of Public Health, http://www.fhi.no.

Antimicrobial resistant bacteria (Chapter 5)

Literature regarding antimicrobial resistant bacteria obtained using the following criteria about bacteria, resistance, matrix, and Geographical and technology relevance, in PubMed. The search was done 29.11.2019 with no limitation of time period.

Bacteria:

1. Gram-negatives: *Enterobacteriaceae* OR coliform* OR *E. coli* OR *Klebsiella* [All Fields]
2. Gram-positives: enterococc* [All Fields]

Resistance:

3. Gram-negatives: resistance OR tet OR tetracycline OR *quinolone OR qnr OR sul OR sulfonam* OR MAR-index
4. Gram-positives: Van OR Vancomycin OR Erm OR erythromycin OR resistance [All Fields]
5. Emerging resistances: ESBL OR Carbapenemase OR pAmpC OR CTX-M OR OXA OR NDM OR cephalosporin* OR colistin* OR MCR OR MDR [All Fields]

Matrix:

6. Water phase: wastewater OR sewage [title/abstract]
7. Solid phase: sludge [title/abstract]

Geographical and technology relevance:

8. Europe OR Scandinavia OR Norway OR Sweden OR Finland OR Denmark OR Netherlands OR Germany [All Fields]

Inclusion criteria: Enumeration of specific resistance or frequency resistant of enumerated, bacterial species, genera or family before and after wastewater or sludge treatment.

Chapter	Search	Hits	Full text used	Fulfilling criteria
5.1.1	1 * 3 * 6 * 8	117	17	13
5.1.2	2 * 4 * 6 * 8	48	10	7
5.1.3	1 * 5 * 6 * 8	56	11	4
5.1.5	(1OR2)*(3OR4OR5)*7*8	220	23	1

Antimicrobial resistance genes (Chapter 5)

Literature search regarding ARG was performed (March 2019-Jan 2020) in PubMed and Google Scholar using the following search terms and combinations thereof: resistance genes, ARG, wastewater, sewage, sludge, treatment. Ninety-tree articles were qualified to be included on this topic.

Antimicrobial agents in sewage discharged, sludge and soil (Chapter 4.4)

Data regarding antimicrobial agents in sewage discharged, sludge, and soil in Norway was obtained from the following reports, regulation and reports and relevant articles:

Reports from Norsk Vann (Norskvann, 2009; Norskvann, 2017; Norskvann, 2020).

Data and information from Chemical database (Norwegian Environmental Agency).

NIVA 2017; Riverine Inputs and Direct Discharges to Norwegian Coastal Waters – 2016, NIVA Report 7217/Miljødirektoratet M862, 206 pp) (Skarbøvik et al., 2017).

Lovdata: Forskrift om gjødselvarer mv. av organisk opphav (Gjødselvareforskriften – «Norwegian fertilizer regulation»). FOR-2003-07-04-951. published 01.01.2003. last modification FOR-2019-01-30-58. https://lovdata.no/dokument/SF/forskrift/2003-07-04-951 (last accessed 15.06.2020), (Lovdata, 2003).

Other relevant articles.

Wastewater and sewage treatment (Chapter 4.4)

Data and information regarding wastewater and sewage treatment obtained from the following sources:

The Norwegian "forurensningsforskriften" [reference:
https://lovdata.no/dokument/SF/forskrift/2004-06-01-931

(Lovdata, 2004).

VKM 2009 (Sundstøl et al., 2009).

Lov om vern mot forurensninger og om avfall (forurensningsloven), LOV-1981-03-13-6. issued 01.10.1983; last modification 01.11.2019. ISBN 82-504-1304-0. https://lovdata.no/dokument/NL/lov/1981-03-13-6 (last access 19.04.2020) (Lovdata, 2019).

Forskrift om begrensning av forurensning (forurensningsforskriften), FOR-2004-06-01-931. issued 01.07.2004; last modification 01.01.2020. https://lovdata.no/dokument/SF/forskrift/2004-06-01-931?q=forurensningsforskriften (last access 19.04.2020) (Lovdata, 2004).

Other relevant articles.

In addition to the data and articles used in this section, the expert's (Wolfgang Uhl) knowledge and experience regarding different treatment methods and experience in the field was essential for the topic.

Exposure assessment and Characterization of the probability for development and dissemination of AMR via effluent wastewater and applied sludge (Chapter 7)

Literature used in these chapters were based on the reports and articles referenced in other chapters in this assessment. Reports and articles were scrutinized to identify additional articles or reports that had not been identified by our searches.

2.1.1 Inclusion criteria

Presence of antimicrobial agents, antimicrobial resistant bacteria and antimicrobial resistance genes in wastewater, wastewater effluents, sludge, soil and water environments. HGT in relation to the level of resistance drivers.

2.1.2 Exclusion criteria

Antimicrobial agents used in fish farms, in agriculture (except for agents used in pets like cats, dogs), ARB and ARG due to use in fish farms and agriculture.

2.1.3 Relevance screening

The titles of all hits were scanned, and for those that were of potential relevance, the abstracts were also inspected. The relevance screening was performed independently by every member of the working group. Citations were excluded if they did not relate to the terms of reference. Reference chasing was used to identify additional articles or reports that had not been identified by our searches.

3 Problem identification

THE NFSA and NEA writes in their assignment to VKM the following: "The prevalence of ARBs and ARGs is expected to be higher in environments as wastewater treatment plants (WWTPs) as they serve as important reservoirs receiving wastewater from household, industry and hospitals where antibiotics are applied. Wastewater from WWTPs could constitute a source for spread of AMR into the environment, and the assessment should consider its risk".

The structure for a classic risk assessment of the assignment would be to identify and characterize the hazards related to AMR development and dissemination from WWTPs and into the environment, describe the probability of human exposure to AMR from different environments and food chains, and assess the consequences for human health arising from this exposure. The bottom-line question would to assess whether there is an increased risk for humans becoming infected with resistant pathogenic bacteria due release of ARB, ARG and RDs from WWTPs and into different environmental compartments. As there are so many uncertainties (chapter 11) and data gaps (chapter 13) associated with an assessment of the consequences for human health, this opinion deviates in its structure from the classic risk assessment.

AMR in WWTP can be understood as a problem caused by:

1. The direct effect caused by the presence of ARB in effluent wastewater and applied sludge that eventually end up in the food chain.
2. The indirect effect arises through selection and increased abundance of ARB and/or ARG in effluent wastewater and applied sludge, due to simultaneous presence of antimicrobial agents selecting for AMR development and dissemination. The agents may exert their effects in untreated WW, but also in effluent and applied sludge. Due to the presence of resistance drivers and opportunities for horizontal gene transfer, previously susceptible bacteria may become resistant to antimicrobial drugs, and such bacteria may end up in the food chain.

Thus, ARB and/or ARG may be recycled into food chains from their occurrence, or emergence in applied sludge or WW effluent. Certain groups of people may also be directly exposed to ARB and/or ARG through their work with WW and/or applied sludge.

NFSA and NEA emphasize that AMR needs to be seen in a One Health perspective. For this opinion, the One Health concept is regarded as a worldwide strategy for expanding interdisciplinary collaborations and communication in all aspects of healthcare for humans, animals, and the environment. This concept has been adopted as a framework to combat some of the grand challenges of our time, namely the emerging and re-emerging infectious diseases and the increase in development and dissemination of ARB and ARG. The zoonotic character of many emerging infections and AMR emphasizes that human and animal health are inextricably linked. Sewage, WW, and sludge are environmental compartments where

environmental microbiota meet and interact with human (and animal) host microbiota. The dynamics regarding the evolution and spread of AMR within these mixed bacterial populations is complex and not yet fully understood. An important factor is the co-presence of antimicrobial agents that can act as resistance drivers.

4 Background information

4.1 General remarks

This chapter concerns antimicrobial agents and microorganisms in more general and factual terms, such as, for example, mechanisms of action and mechanisms of resistance. Data on the use of antimicrobial agents for humans in Norway are presented, as well as data on the stability of antimicrobial agents in WW. Furthermore, sewage treatment methods related to types of WW, purpose and levels are described, as well as treatment methods and requirements for use of sewage sludge. A separate paragraph describes treatment of hospital WW.

4.2 Antimicrobial agents

4.2.1 Mechanisms of action of antimicrobial agents

4.2.1.1 Types of antimicrobial agents

Antimicrobial agents include antibacterial (antibiotics) and antifungal agents, potentially toxic metals (PTM), and biocides like disinfectants agents. Whereas the modes of action of antibacterial and antifungal agents are based on the effect on one target, the mechanisms of action of PTM and biocides (disinfectant agents) are based on multiple targets or general toxicity in bacteria.

4.2.1.2 Antibiotics

Different antimicrobial agents have different modes of action that follow one or several of the following pathways (Figure 4.2.1.2-1): 1. Inhibitors of cell-wall synthesis. 2. Inhibitors of cell-membrane function. 3. Inhibitors of protein synthesis. 4. Inhibitors of nucleic-acid synthesis. 5. Inhibitors of other metabolic processes. 6. Destruction and/or inhibition of cytoplasmic membrane structure. For further information see (Tronsmo et al., 2016; Wasteson et al., 2017; Yazdankhah et al., 2015).

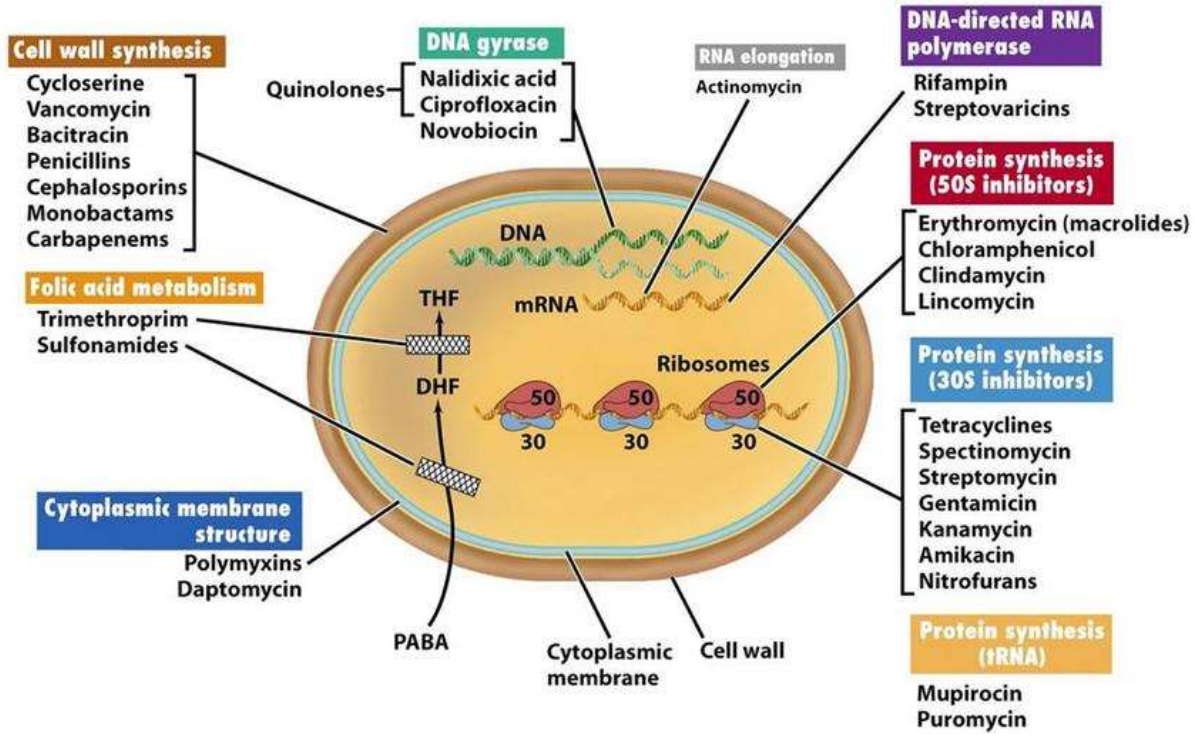

Figure 4.2.1.2-1. The targets for commonly used antibacterial agents. Key: PABA: Para-aminobenzoic acid; DHF: Dihydrofolate; THF; Tetrahydrofolate (Madigan, 2006). Copy allowed from VKM repot: Assessment of antimicrobial resistance in the food chains in Norway (Yazdankhah et al., 2015).

4.2.1.3 Antifungal agents

There are five main classes of antifungal medications; *azoles and polyenes* that inhibit/interact with ergosterol (the main fungal sterol); *echinocandins* that inhibit formation of glucans in the fungal cell wall, *allylamine*s that disrupt squalene oxidase in the fungal cell membrane, and *5-fluorocytosine*, a nucleoside analogue that inhibits nucleic acid synthesis. In addition, griseofulvin that inhibits fungal cell division and ciclopirox (unclear mechanism) are available (Brunton et al., 2019; FHI, 2019).

Fig 4.2.1.3-1 shows the target mechanism and Table 4.2.1.3-1 lists the antifungals that have been available on the Norwegian human medicine market since 1970.

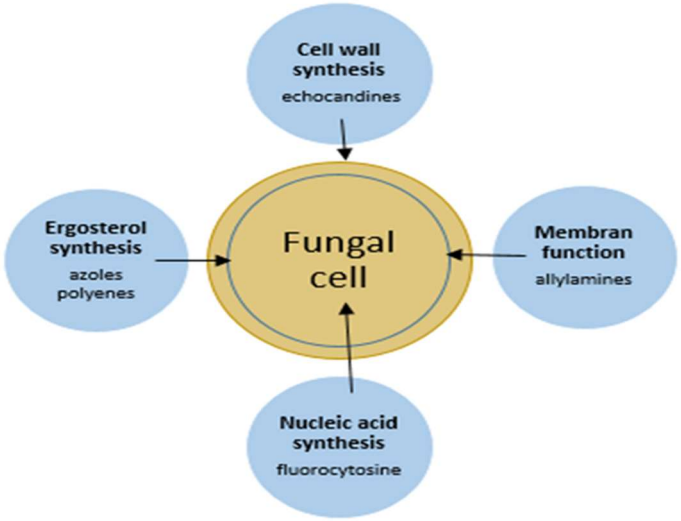

Figure 4.2.1.3-1.

Schematic overview of the mechanisms of action for antifungal groups available for medical purposes in Norway. The arrows mark the sites of action in the fungal cell (Illustration by Hege S. Blix)

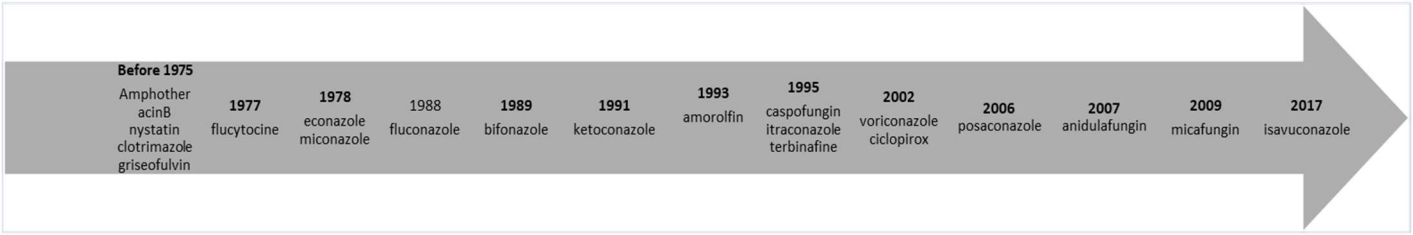

Figure 4.2.1.3-2. Antifungals and the year available in the Norwegian market (FHI, 2019).

Table 4.2.1.3-1.

Antifungals available on the Norwegian market (previously and currently) according to main target, mechanism of action, antifungal group, ATC code, and drug administration route (Brunton et al., 2019; FHI, 2019)

MAIN ACTION TARGET	ANTIFUNGAL GROUP	MECHANISM OF ACTION	ATC CODE	SUBSTANCE	ROUTE OF ADMINISTRATION P=PARENTERAL O=ORAL
CELL WALL SYNTHESIS	echinocandine	Inhibits (1,3)-β-o-glucan synthase	J02AX06	anidulafungin	systemic; P
			J02AX04	caspofungin	systemic; P
			J02AX05	micafungin	systemic; P
CELL MEMBRANE	azole	Inhibits ergosterol synthesis causing accumulation of toxic sterols which that membrane stress and inhibit growth of the fungi	D01AC01/ G01AF02	clotrimazole	local
			D01AC03/ G01AF05	econazole	local
			J02AC01	fluconazole	systemic; P
			J02AC05	isavuconazole	systemic; O,P
			J02AC02	itraconazole	systemic
			J02AB02/ D01AC08	ketoconazole	systemic; O and local
			D01AC02/ A01AB09	miconazole	local
			J02AC04	posaconazole	systemic; O,P

			J02AC03	voriconazole	systemic; O,P
			D01AC10	bifonazole	local
	polyene	Binds to ergosterol in fungal cell membrane and increases membrane permeability	A07AA02	nystatin	local
			J02AA01	amphotericin B	systemic; P and local
	allylamine	Inhibits squalene epoxidase and prevents ergosterol synthesis	D01BA02/ D01AE15	terbinafine	systemic; O and local
NUCLEIC ACID SYNTHESIS	Other	Fluorinated pyrimidine that interrupts nucleic acid and protein synthesis	J02AX01	flucytosine	systemic; P and local
FUNGAL CELL DIVISION		Inhibits fungal cell division by disruption of the mitotic spindle structure	D01BA01	griseofulvin	systemic; O
OTHER		Hydroxypyridone, unclear mechanism	D01AE14	ciclopirox	local
OTHER		Morpholine derivative, interferes with sterol synthesis	D01AE16	amorolfin	local

4.2.1.4 Potentially toxic metals (PTM)

In a metal, atoms readily lose electrons to form cations that are surrounded by delocalized electrons. This behaviour is responsible for the conductivity and antimicrobial effects of metals (Fraise et al., 2012). Microbial toxicity may be due to the chemical affinity of PTM for thiol groups of macro-biomolecules, but also depends on the solubility of the metal compounds under physiological conditions (Lemire et al., 2013). Several possible modes of action of PTM have been reported: a) protein dysfunction; b) production of reactive oxygen species (ROS) and antioxidant depletion; c) impaired membrane function; d) interference with nutrient uptake; and e) geno-toxicity.

Specific mechanisms of action for different PTM (arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc) in bacteria have previously been discussed in a VKM opinion (Wasteson et al., 2017).

Copper-coated surfaces are used in some clinics to reduce the risk of nosocomial infections (Grass et al., 2011), and both copper and zinc are among the PTM that are known to exhibit cross-resistance with antimicrobials, such as glycopeptides/macrolides and methicillin, respectively. As a result, these elements can play an indirect role in the selection for ARB. In general, even when no antimicrobial compounds are used, certain heavy metals can maintain, or even increase, bacterial resistance against certain agents. The selective pressure exerted by Cu- and Zn-containing materials may contribute to maintaining low levels of these resistant bacteria in environmental microbiota. At re-exposure to glycopeptides or macrolides, the resistant bacteria may rapidly proliferate and become a dominant part of the microbial population.

4.2.1.5 Biocides (disinfectants)

In contrast to chemotherapeutic agents, biocides (in particular disinfectant agents) have multiple target sites within the microbial cell. The overall damage to these target sites results in the **bactericidal effect**. **Bacteriostatic effects**, usually achieved by a lower concentration of a biocide, might correspond to a reversible activity on the cytoplasmic membrane and/or the temporal impairment of enzymatic activity. The bacteriostatic mechanism(s) of action of a biocide is less documented and a primary target site within the cell might be involved (Maillard, 2002).

Specific mechanisms of action for different disinfectant agents have previously been discussed in a VKM opinion regarding AMR due to the use of biocides and heavy metals (Tronsmo et al., 2016).

According to the EC Product Directive 98/(/EC (BPD) (EU, 1998), which was adopted by the European parliament in 1998, biocides are classified into four main groups according to their application categories and further sub-divided into 23 product groups (Tronsmo et al., 2016).

In this opinion, we focus on biocides with potential antibacterial activity and their ability to induce AMR in bacteria. These are largely products that belong to main group 1, disinfectants. This group includes products used in human hygiene, veterinary hygiene, water treatment, and products used in the food and feed area, like phenols (triclosan), alcohols, aldehydes, anilides (talicylanilides, carbanilides), peroxygens (hydrogen peroxide, peracetic acids), biguanides (chlorhexidine, alexidine, polymeric biguanides), QACs (quaternary ammonium compounds), organic and inorganic acids, acridine, triphenylmethane, quionones, and diaminides.

Among these products, phenols (e.g., triclosan), biguanides (chlorhexidine, alexidine, polymeric biguanides), and QACs will be discussed further, because of their common use and their ability to induce resistance and co- or cross-resistance to other antimicrobial agents in bacteria. Other products either do not result in the development of resistance (alcohols, peroxygens, organic/inorganic acids) or are not widely used (aldehydes, anilides, acridine, triphenylmethane, quionones, and diaminides).

4.2.1.6 Others

Several compounds, not primarily used for their antimicrobial activity, may nevertheless occur in sewage. The levels of such compounds may be relatively high, and although the compounds have low inhibitory effect (low MIC value), they may contribute to the stresses met by bacteria in sewage. Stressed bacterial cells can more readily attract or accept laterally transferred genes, including ARG (Baharoglu et al., 2013).

Food ingredients from plants may have an impact on gut microbiota and, thus, also on sewage microbiota (Hintz et al., 2015). Phytochemicals, such as flavonoids, quinones, tannins, glycosides, and essential oils, and antimicrobial peptides, such as thionins and plant defensins, will be parts of the microbial stress factors in sewage. The mechanisms of action from various plant-derived antimicrobial compounds are very heterogenic and depend on the group of molecules; for several molecules, the mechanism of action is still unknown (Borges et al., 2015). Examples of mechanisms of action are the impact of the cell permeability of thionins, increasing uptake of isoaminobutyric acid and changing the flux of calcium and potassium ions across the microbial membranes. Flavones and flavonoids bind to many bacterial proteins and disturb their activity, including proteins in the cell walls of the microbes. The concentrations of plant-derived antimicrobial compounds in sewage have not been studied (Cowan, 1999; Sakkas and Papadopoulou, 2017).

4.2.2 Use of antimicrobial agents in human medicines in Norway

4.2.2.1 Antibiotics

In 2018, the overall sale in Norway of antibacterials, antifungals, and drugs for tuberculosis for use in humans, measured in weight of active substance, was 36 tonnes (Figure 4.2.2.1-1). This was approximately the same as in 2006. Antifungals and drugs for tuberculosis are

not much used and the antibacterial share has been stable for several years, 96% of total. In 2018, the weight of antibacterials used amounted to 35 tonnes.

Total sales data are captured from the Norwegian drug wholesales statistics, which includes all sales of medicines in Norway, prescribed as well as over-the-counter (OTC). We have included antimicrobials that are used as medicines for humans and not antimicrobials for animals, because human use is the more plausible source of what occurs in WW. However, there are other possibilities for antimicrobials in WW; e.g., medicines can be purchased abroad and used in Norway. However, this is probably a small amount, as there are legal restrictions on substances regarded as medicines, and import should be reported to the authorities. Some antibacterials and antifungals could also be used for industrial purposes, but this probably only small-scale use (e.g., in 2018, 0.13 kg of bronopol, an antifungal agent, was reported to be used for preservation and testing of milk samples). In contrast, bronopol is used in much larger amounts in fish farming, and this is reported and captured in the total sales data; in 2018 this use accounted for 1.1 tonnes. How much of this amount will end up in WW is unknown.

All drug formulations that include antimicrobials are included in the total count; that is systemic preparations, such as oral and parenteral forms, and local preparations, such as for eye, ear, or dermatological uses. Methenamine, an oral prophylactic agent for urinary tract infections (UTI), is not included, because the mechanism of action is through ammonia and formaldehyde, and methenamine is not regarded as being a driver of resistance.

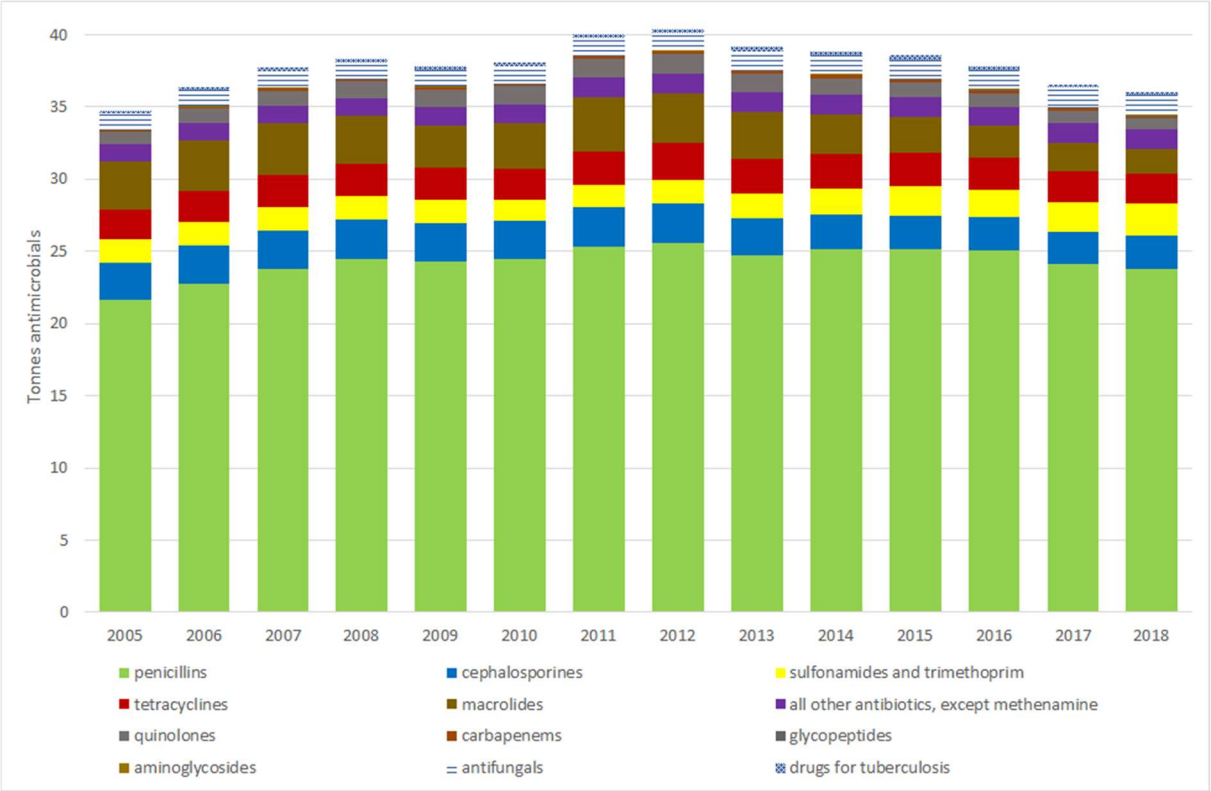

Figure 4.2.2.1-1. Sales, in tonnes of active substance, of antimicrobials for human use, for the years 2005-2018. Antifungals and drugs for tuberculosis are shown at the top (blue/white texture). (NORM/NORM-VET, 2018).

Penicillin is highly used in humans in Norway (green colour on figure), accounting for 66% of the total antimicrobial weight in tonnes. Narrow-spectrum penicillins (penicillins V and G), including all substances in ATC code J01CE, account for 39% of the total volume of antimicrobials used in 2018. However, their share of the penicillin group has declined from 72% in 2006 to 59% in 2018. One antibacterial substance, phenoxymethylpenicillin (penicillin V), accounted for 40% of the weight of the antibacterials; (12.3 tonnes, 34% of total antimicrobials) and one antifungal, terbinafine, accounted for 45% of the weight of the antifungals.

Oral formulations dominate in human medicine, with 86% of the mass of antibacterials used for humans in 2018 being oral forms followed by parenteral formulations (13%). Sales of other formulations, for eye, ear, and skin, are limited. The use of antimicrobial agents in hospitals is more broad spectrum than in ambulatory care. Thus, WW from hospitals will include high-risk antimicrobials with regards to driving AMR. In Table 4.2.2.1-1, the final column shows the hospital share of total sales of antimicrobials, and demonstrates that the critically important antibiotics, such as cephalosporins, carbapenems, glycopeptides, are highly utilized in hospitals (WHO, 2019).

Table 4.2.2.1-1. Total amount in kilograms (kg) of antimicrobials sold on the Norwegian market for human consumption in 2006 and 2018. Proportional change (%) in sales from 2006-2018. The share (%) of consumption attributed to the use in hospitals is presented for 2018.

	Class of antimicrobials	**2006 (kg)**	**2018 (kg)**	**Proportional change 2006-2018 (%)**	**Hospital use, by sales in 2018 (%)**
Antibacterials	Narrow- spectrum penicillins	16393	14011	- 15	8
	Other penicillins	6348	9805	+ 54	11
	Cephalosporines	2678	2282	- 15	66
	Sulphonamides & trimethoprim	1582	2249	+ 42	7
	Tetracyclines	2178	2014	-8	2
	Macrolides	3519	1734	-51	6
	Quinolones	975	774	- 21	10
	Carbapenems	119	142	+ 19	90
	Glycopeptides	30	67	+ 127	93
	Aminoglycosides	38	46	+ 22	86
	Other antibacterials	1186	1309	+ 10	NA
Antifungals	Azoles	566	594	+ 5	15
	Other antifungals	523	766	+ 47	14
Drugs for tuberculosis (TBC)	Rifampicin	66	76	+ 15	NA
	Other TBC-drugs	150	124	-17	NA

NA=no data available

The sales of antibacterial medications reduced from 2006 to 2018, the sales of anti-tuberculosis drugs remained stable, and sales of antifungals have increased.

4.2.2.2 Antifungals

In addition to their function as therapeutic agents, azole fungicides can be used in household products such as shampoos, dermal creams, soaps, toothpastes, and shower gels. For instance, ketoconazole is used as an anti-dandruff agent in haircare formulations with a proportion of approximately 2% (Wishart et al., 2008). Climbazole is applied not only as an antidandruff active ingredient, but also as an antimycotic preservative or an anti-aging

agent, with its content up to a maximum concentration of 2% in rinse-off products, 0.5% in leave-on products, and 0.5% in cosmetic products, respectively (Scientific Committee on Consumer Products, 2019). The usage of climbazole in the European Union (EU) is reported to be in the range of 100–1000 tonnes per annum, which is the second largest usage category (ECHA, 2013). After application of azole fungicides, the residues of these agents may reach the receiving environment via direct or indirect discharge of WW, thus posing potential risks to non-target organisms.

Some antifungals are used for industrial purposes (e.g., bronopol), for which 0.13 kg was reported to be used for preservation and testing of milk samples in 2018. On the other hand, bronopol is used in much larger amounts in fish farming, and in 2018 this accounted for 1.1 tonnes. How much of this amount that will end up in wastewater, is not known.

4.2.2.3 Potentially toxic metals

PTMs occur ubiquitously in the environment, and, on occasions, at high concentrations in certain settings. The most commonly encountered PTM contaminations in order of abundance are zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), silver (Ag) and mercury (Hg) (Wuana and Okieimen, 2011). Certain products containing PTM, such as zinc (Zn) and copper (Cu), are used in agriculture for various purposes. In 2017, the EU decided that veterinary use and feed addition of (zinc oxide) ZnO will be banned within 2022 to prevent undesirable environmental effects.

Table 4.2.2.3-1. Domestic and imported PTM used in Norway

Metals	Total domestic metal trade in Norway, 2017 (tonnes)	Imported metals, 2017 (tonnes)	Produced metals, 2017 (tonnes)
Pb	16370	16369	1.6
Hg	0.002	0.002	0
Ni	52149	52170	86495
Ag	1.5	1.5	0.5
Cd	21.3	1.3	20.3
Co	745	745	3473
Cu	59705	59705	1.3
Zn	24656	24183	9207
Source: Norwegian Environmental Agency (Miljødirektoratet)			

4.2.2.4 Biocides (disinfectants)

Disinfectants are extensively used, and their formulations contain active ingredients at levels well above the minimum inhibitory concentration (MIC) of target microorganisms. Inappropriate application of disinfectants, dilution in the environment after discharge, and biodegradation result in biocide concentration gradients (Tezel and Pavlostathis, 2015).

According to data provided by the Norwegian Environmental Agency, approximately 14000 tonnes of disinfectant agents (types 1, 2, 3, 4 or 5) were used in 2015 (PT1 human hygiene, PT2 disinfectants and algaecides not intended for direct application to humans and animals, PT3 Veterinary hygiene, PT4 Food and feed area, PT5 drinking water). The most commonly used disinfectants in Norway, and at the highest volume, are: ethanol, sodium hypochlorite, propan-2-ol, propan-1-ol, QAC, H_2O_2, peracetic acid, pentapotassium bis(peroxymonosulphate) bis(sulphate), glutaral, and 2-phenoxyethanol (personal communication, Espen Wigaard) (Tronsmo et al., 2016).

Although most disinfectant agents are known to be high-volume products, it was not possible to gather exact data regarding the use of different active substances in Norway within the disinfectant agents included in this report. Data regarding the concentrations of disinfectant agents in WW and sludge in Norway are lacking.

4.2.2.5 Others

No information/data was found.

4.2.3 Stability of antimicrobial agents in the environment

4.2.3.1 General remarks

Antimicrobial agents are not completely metabolised in humans and animals. They are excreted via urine and faeces, either as parent compounds, or as metabolites. They may enter the environment directly along with the faeces (e.g., through land application of manure), or by being discharged to sewage systems and, possibly after sewage treatment, via discharge of treated effluent (Bondarczuk et al., 2016).

There is no systematic monitoring of antimicrobial residues in WW in Norway. Sporadic measurements have been made related to specific research projects and other assignments, and some of these are referenced in this opinion.

The following table provides an overview about the stability of antibiotics for medication of humans and thus about the ratio of antibiotics discharged to sewage.

Table 4.2.3.1-1. Some pharmacokinetic properties of antibacterials used in Norway in 2019.The selected substances accounts for 85% of the DDDs and more than 80% of the weight of human antibacterials per year. Information collected from SPCs (summary of products characteristics) and Martindale (Micromedexsolutions, 2020)

ATC-group	Substance	Serum half-life (h)	Pharmacokinetics
J01A - Tetracyclines	Doxycycline	16-22	Partly inactivation in the liver. Renal excretion: about 40%. Mainly excreted as inactive chelate in faeces.
	Lymecycline	10	Metabolized to tetracycline. Mainly excreted in the urine (60%) and mainly unchanged.
J01CA - Penicillins with extended spectrum	Amoxicillin	1	Metabolized to a limited extent. About 60% is excreted unchanged in the urine some may be excreted in the faeces.
	Pivmecillinam	1	Metabolized to a limited extent. Renal excretion about 50%, some is excreted in bile.
J01CE - Beta-lactamase sensitive penicillins	Benzylpenicillin	0.5	Metabolized to a limited extent; around 20 %. Renal excretion>60% (as parent drug).
	Phenoxymethyl penicillin	1	Metabolized in the liver to a greater extent than benzylpenicillin (around 55 %). Unchanged drug and metabolites are excreted in the urine.
J01CF - Beta-lactamase resistant penicillins	Dicloxacillin	0.5	Metabolized to a limited extent, around 10%. Renal excretion>60% (parent drug).
J01DD – Third gen. cephalosporines	Cefotaxime	1	Metabolized in the liver to active and inactive metabolites. Renal excretion 40 to 60% unchanged, about 20% in the faeces.
J01E - Sulfonamides and trimethoprim	Trimethoprim	8-10	Metabolized in the liver, 10 to 20%. Renal excretion around 50% (parent drug), small amounts, 5%, are excreted in the faeces.

	Sulfamethoxazole and trimethoprim	11	Trimethoprim, see above, Sulfamethoxazole: Metabolized in the liver, mainly to inactive substances. Renal excretion around 20% (parent drug).
J01FA - Macrolides	Erythromycin	1,5-3	Metabolized in the liver to mainly inactive metabolites. Mainly hepatic excretion (feces), renal excretion 10%.
J01MA - Fluoroquinolones	Ciprofloxacin	4-7	Metabolized to several metabolites with somewhat lower antimicrobial activity Renal excretion 40-50% (parent drug). Faeces 25% (parent drug).
P01AB – Nitroimidazole derivatives	Metronidazole	8	Metabolized in the liver to with antibacterial activity. Mainly renal excretion, a small amount appears in feces.

4.2.3.2 Antibiotics

Antibacterial agents are a diverse group of organic chemicals. Almost all (naturally, semi-synthetic, and synthetic antibiotics) consist of a non-polar core combined with polar and ionisable functional groups (Thiele-Bruhn, 2003).

Generally, antibacterial agents (antibiotics) carry a negative, positive, or both negative and positive charge at environmental pH. The polar and/or ionisable groups make the antibiotics rather soluble in water. The solubility of the antibacterial agents (antibiotics) range from the sub-mg/L level (e.g., hydrophobic macrolides) to several or even hundreds of grams per litre (e.g., aminoglycosides and the polar amphenicols). In addition, there is a high variation in solubilities within antibiotic classes (Schwarzenbach et al., 2003).

Antibacterial agents are susceptible to multiple modes of degradation in the environment, as shown in Table 4.2.3.2-1.

Table 4.2.3.2-1. Antibacterial susceptibility to modes of removal or degradation from the environment for some class of antibiotics (Morris, 2015).

Removal/degradation	Penicillins	Macrolides	Cephalosporins	Tetracyclines	Sulphonamides	Quinolone/ fluoroquinolones
Photolysis	X	X	X	Y	Y	Y
Hydrolysis	Y	–	Y	Y	Y	X
Thermolysis	Y	–	–	–	Y	X
Sorption[a]	Y	Y	–	Y*	Y	Y
Biodegradation	Y	X	X	X	X	X
Anaerobic conditions	–	–	–	–	Y	X

'–' indicates that data were not available.
a Removes antimicrobials from the water by attachment to particles, but this does not mean it has been degraded.
X, not susceptible; Y, susceptible; Y*, susceptible but weak.

The environmental residual concentrations of antibacterial agents are not only due to their continuous release into the environment, but to their intrinsic high persistence as well. Some antibacterial agents, like penicillins, are easily degraded, while others, like fluoroquinolones (e.g., ciprofloxacin), macrolides (e.g., azithromycin), and tetracyclines are considerably more persistent, resulting in them remaining longer in the environment, spreading more widely, and accumulating in higher concentrations (Blackwell et al., 2005; Hamscher et al., 2002; Lin et al., 2010). Sulphonamides are susceptible to many forms of degradation (Morris, 2015).

4.2.3.3 Antifungals

Azole fungicides are widely detected in surface waters and sediments of the aquatic environment due to their incomplete removal in WWTP. These chemicals are resistant to microbial degradation, but can undergo photolysis under UV irradiation. Due to different physiochemical properties, azole fungicides show different environmental behaviours (Chen and Ying, 2015).

There are three major mechanisms that may influence the fate of antifungal agents in the environment: hydrolysis, photolysis, and biodegradation. Azole fungicides do not appear to undergo **hydrolysis**. Fluconazole is stable in aqueous solution for three days (Chen et al., 2013). Clotrimazole is hardly degradable in either alkaline (pH 9) or neutral solutions (pH 7), but hydrolyses to (2-chlorophenyl)-diphenyl methanol and imidazole in acidic medium (pH 4) with a reported half-life of 20 days (OSPAR, 2005).

The mechanism of **photolysis** is the chemical transformation of a compound via energy and electron transfer reactions induced by light (Zhang et al., 2010). Fluconazole could be transformed to defluorinated hydroxylated by-products in aqueous solution under UV-254 nm, with photolysis half-lives of 85 min at pH 5, 115 min at pH 7, and 27 min at pH 12 (Chen et al., 2013). Similar to fluconazole, the UV photolysis half-life of climbazole also depends on solution pH in the following order: pH 9 (42 min), pH 5 (66 min), pH 7 (70 min) (Couteau et al., 2000). These two azole fungicides are more rapidly photodegraded in alkaline solution than in acidic or neutral solution. Despite their apparent photodegradation

under laboratory conditions, the UV disinfection regimes used in WWTPs and drinking water treatment plants did not produce significant elimination for azole fungicides (Kang et al., 2004; Meunier et al., 2006; Peng et al., 2012).

Azole fungicides used in agriculture are moderately lipophilic and fairly persistent, with typical half-lives of weeks to months (Tomlin, 2003). They are not readily **biodegradable** as predicted by the (EPA, 2020); this is attributed mostly to their inherent characteristics of bacteriostasis to inhibit microbial activity. Under field conditions, many factors (e.g., temperature, moisture, weather, and redox) could affect the dissipation of azole fungicides, so it is often not appropriate to predict the fate of the azole fungicides in field soils. Current data available in the literature suggest high persistence in soil environments (Chen and Ying, 2015).

4.2.3.4 Biocides (disinfectant agents)

Phenols/bisphenols like triclosan and triclocarban have been detected in many environments, such as surface waters (1.4–40,000 ng/L), sediments (<100–53,000 ng/kg d.w.) and, in lower concentrations, in soil after biosolid application (1.5–13 ng/kg). Together with their metabolites they can remain in soil for several years (Boxall et al., 2003; Rivier et al., 2019).

Sewage sludges produced at WWTPs around Norway are infrequently monitored regarding numerous contaminants, including pharmaceuticals, biocides, and heavy metals. **Phenols and bisphenols** measured in 2018 showed a decrease compared to previous years, continuing a trend observed since 1996. Nonylphenol+etoxilates today have median concentrations of 3.8 mg/kg, while bisphenol A has a median concentration of 1.1 mg/kg. Other bisphenols were at less than 10 % of this value. **Organotin compounds** comprise tributyltin and its relatives that have been used as anti-fouling agents, particularly for hulls of smaller boats. Records on these substances are scarce, but sewage sludge from four WWTPs contained from 25-100 µg/kg DM in 2018, representing a 50 % increase since 2012 for 2 of three plants. The general biocide **triclosan** was very variable between WWTPs, with 2 out of 16 having mean sludge concentrations of approx. 4 mg/kg, while the median value was 0.4 mg/kg. The latter represented a 69 % decrease since 2013, indicating that, in general, the use of triclosan has been reduced in recent years (Blytt and Stang, 2018).

Biguanide (e.g. chlorhexidine). According to Environment and Climate Change Canada Health Canada (2017) (Canada, 2017), the available information indicates that chlorhexidine tends to persist in water, sediments, and soil. Half-lives in water and soil are greater than 182 days and are greater than 365 days in sediment. As a result of its persistence, there is a potential for prolonged exposure to chlorhexidine, both close and distant to points of discharge to the environment. There is also the potential for increased spatial exposure in the aquatic environment as a result of its affinity to negatively charged particles and transport via suspended solids and sediment. However, its persistence may allow more time for chlorhexidine to associate with negatively charged particles and therefore become less

bioavailable. Chlorhexidine is expected to have a low potential to bioaccumulate based on its high solubility in water.

QACs (quaternary ammonium compounds). According to the Zhang et al. most industrial and domestic uses of QACs can be expected to lead to their release to WWTPs and thereafter their dispersal into various environmental compartments through sewage effluent and land application of sludge (Zhang et al., 2015a). The contamination levels of QACs in sewage and surface water are in the range of ng/L to µg/kg to mk/kg (dw). Although QACs are considered to be aerobically biodegradable, the degradation is affected by their chemical structures, dissolved oxygen concentrations, complexing with anionic surfactants, etc. A high abundance of QACs has been detected in sediment and sludge samples due to their strong sorption and resistance to biodegradation under anoxic/anaerobic conditions. (Zhang et al., 2015a).

Only sparse data are available concerning the stability, solubility, and biodegradability of QACs. In general, it seems that the biodegradability decreases with increasing numbers of alkyl chains: R(CH3)3N+ > R2(CH3)2N+ > R3(CH3)N+. Within each category, the biodegradability seems inversely proportional to the alkyl chain length. Heterocyclic QACs are less degradable than non-cyclic (Thorup, 2000). Positively charged QACs are mainly sorbed to clay minerals, reducing their acute toxicity, but increasing their persistence (Mulder et al., 2018).

4.2.3.5 Potentially toxic metals (PTM)

As they are not degraded and they persist in the environment, PTM are of concern to public health professionals (Yu et al., 2017)**.** Some metalloids/metals have different oxidation states (As, Cr), where oxidized forms are both less toxic and occur most frequently. Some other metals (Ag, Hg, Pb) are prone to precipitation to insoluble salts or sulphides during wastewater treatment (WWT) and thus transform to non-bioavailable and non-toxic forms (Adams and Kramer, 1999).

In soil, natural background levels are frequently so high that amendments with sewage sludge permitted for agricultural use in Norway constitute a marginal increase in total soil concentrations. Soils also sequester metals to a large extent, reducing their bioavailability and limiting uptake in plants and organisms. Such sequestration also features increasing binding strength with time, and is particularly strong for Pb (>99.9%), but rather weak for Cd (approx. 80-90 %). Other elements experience an intermediate degree of sequestration (approx. 95-99 %).

4.2.3.6 Others

No information is available

4.2.4 Antimicrobial agent residues in wastewater in Norway

4.2.4.1 Antibiotics and antifungal agents

No data regarding systematic measuring antibacterial agents and antifungal agents in WW in Norway were available at the time of this review.

4.2.4.2 Potentially toxic metals

There is no systematic monitoring of PTM in WW in Norway. However, some data are available from a recent PhD project at NMBU. Paulshus et al. measured the monthly concentrations of Cu, Zn, Ni, and Cr at three WW sites in the Oslo region (Paulshus et al., 2019a). The levels were similar at all sites, and no seasonal variation was observed. The concentrations of Cu and Zn in the community wastewater averaged 1.5 µM (range 0.85-2.36 µM) and 2.4 µM (range, 1.32-3.67 µM), respectively, and were about 50-fold higher than those of Ni and Cr.

4.2.4.3 Agents in hospital wastewater

To assess the special situation of hospitals, antibiotic use in society for different purposes and at specific locations must be considered. Figure 4.2.4.3-1 illustrates the use of antimicrobials for animals and humans in Norway and, for comparison, in Germany. For human use, the relative amounts used in hospitals and in outpatients is shown.

The total use of antibiotics is related to the number of inhabitants and to the number of hospital beds. The population of Germany is about 83 million, and thus the consumption of antibiotics for human use is about 9.1 g per inhabitant per year. For Norway, with a population of about 5.5 million (Statista, 2020b), the respective consumption is about 6.5 g per inhabitant per year. Taking into account the number of hospital beds in Germany, which is 8 per 1,000 inhabitants, or about 0.8 % (Statista, 2020a), and in Norway about 3.9 per 1,000 inhabitants (0.39 %) (Tradingeconomics, 2020), about 300 g antibiotics per hospital bed per year are used in Germany, while about 150 g per hospital bed per year are used in Norway.

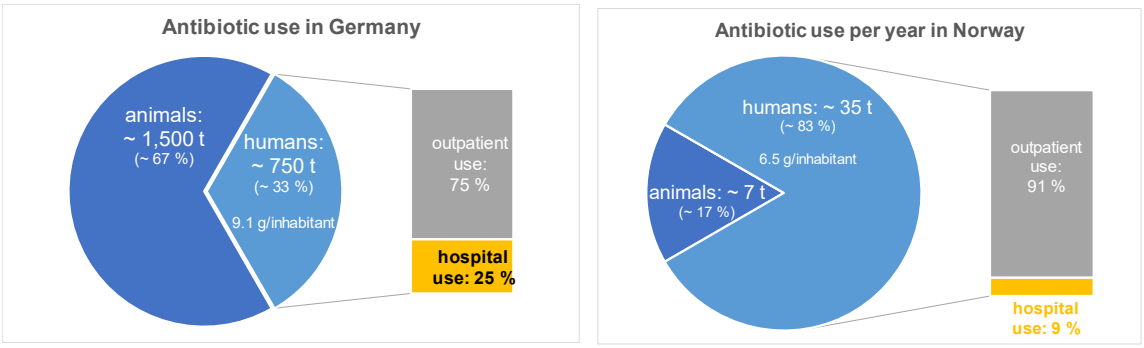

Figure 4.2.4.3-1. Antibiotic use in tonnes per year in Germany and Norway. Antibiotic use data for humans for Germany from 2015 (GERMAP, 2015), from animals from 2018 (Fleischatlas, 2018). Illustration by Irene Slavik, with kind permission.

Assuming that all hospital beds are more or less permanently occupied, and that WW from hospitals per bed amounts to approximately three times the amount produced per person in households (Ødegaard et al., 2009), it can be calculated that the mass-concentrations of antibiotics in hospital WW in Germany will, on average, be about 14 times as high as the concentrations in sewage from households. For Norway, it is calculated that antimicrobial concentrations in hospital WW should be about 9 times as high as in sewage from households.

4.3 Microorganisms in wastewater

4.3.1 General information

As described in the introduction, bacteria belonging to the human and animal microbiota are released into WW, which is a new environmental compartment and exposes them to novel environmental conditions to which they must adapt. In this compartment, these bacteria will have to compete with the indigenous population for survival and growth. Studies have shown that microbial sewage communities represent a combination of inputs from human faecal microbes and enrichment of specific microbes from the environment to form a unique population structure. A study from McLellan et al. describes how the profile of untreated sewage in a US metropolitan area included a discernible human faecal signature of several taxonomic groups (several species within *Bifidobacteriaceae*, *Coriobacteriaceae*, *Bacteroidaceae*, *Lachnospiraceae* and *Ruminococcaceae*) (McLellan et al., 2010). However, the faecal signature made up a small fraction of the taxa present in sewage. Beta- and Gammaproteobacteria were much more abundant, indicating that bacteria from these groups proliferated in the sewage system. Others have also shared similar observations. Newton et al. found that about 15% of sewage influent sample sequences could be attributed to human faecal origin, but they also concluded that the sewage recaptured most (97%) of the human faecal oligotypes (genetics, taxonomy) (Newton et al., 2015). Guo et al. found in their study of WW influents, that only 7.3 % of 16S rRNA sequences were shared with the human gut microbiota and 21.7 % with soil microbiota (Guo et al., 2019). The majority of the sequences were associated with bacteria from sewer biofilms and sediments. Guo et al. also showed that sewage microbial communities were active in carbon and nutrient removal activities (Guo et al., 2019). A recent study from McLellan and Roguet showed that *Arcobacter* spp., *Acinetobacter* spp. and *Aeromonas* spp. are among the dominant microorganisms in sewage influent and should be regarded as sewage-pipe residents (McLellan and Roguet, 2019). These bacteria apparently play important roles in biotransformation of waste, but it is important to note that *Acinetobacter* spp. and *Aeromonas* spp. are known to be prone to develop multidrug resistance (MDR) (Zhang et al., 2009).

4.3.1.1 Some specific bacterial pathogens that may occur in wastewater

Campylobacter is considered to be the most common bacterial cause of human gastroenteritis in the world (www.who.org). Campylobacteriosis is not usually treated with antibiotics, but may be treated in the case of severe infections in the elderly and patients with impaired immune systems. (https://www.helsedirektoratet.no/retningslinjer/antibiotika-i-sykehus/abdomen/gastroenteritt).

At a global level, *Salmonella* spp. is the second most common cause of foodborne bacterial infections in humans. Such infections are not usually treated with antibiotics, but in the case of severe infections in the elderly and patients with impaired immune system, an effective antibiotic treatment can be lifesaving (Helms et al., 2004). *Escherichia coli* is a commensal

bacterial species present in the intestine of warm-blooded animals and has traditionally been used as an indicator of faecal contamination in foods and in aquatic environments, including WW (Cotruvo, 2017). However, some strains may cause diseases in humans. Intestinal pathogenic *E. coli* include shigatoxin-producing, enterotoxin-producing, enteropathogenic, enteroaggregative, diffusely adhering and enteroinvasive strains (STEC, ETEC and EPEC, EAEC, DAEC, EIEC, respectively) that are seldom treated with antimicrobials (Helsedirektoratet, 2020). Infections caused by extra-intestinal pathogenic *E. coli* (ExPEC) strains range from UTIs to meningitis and septicaemia, and antibiotic treatment is necessary in many cases (Kaper et al., 2004). The emergence of antibiotic-resistant ExPEC strains has complicated medical treatment of these infections (Pitout, 2012) (see further 5.1.3). *Klebsiella pneumoniae* represents another *Enterobacteriaceae* commensal species of importance for hospital infections and is a major source for antibiotic resistance (Navon-Venezia et al., 2017). Only one study on *Klebsiella* spp. removal during WWT under relevant conditions was found in the literature, and did not indicate selection of *Klebsiella* spp. in WWT (Verburg et al., 2019).

The two Gram-positive agents in the ESKAPE-genera of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.) causing healthcare-associated infections are Enterococci and Staphylococci. Enterococci are members of the intestinal microbiota in a wide variety of hosts. Because of their abundance in faeces of warm-blooded animals and their long-term survival in the environment, they have traditionally been used as indicators of faecal contamination in the aquatic environment, including WW (Taucer-Kapteijn et al., 2016). In the 1970s, they emerged among the leading causes of hospital-acquired, MDR infections. Hospital-adapted pathogenic isolates are characterized by the presence of multiple mobile elements conferring antibiotic resistance, as well as pathogenicity islands, capsule loci, and other variable traits (Gilmore et al., 2013). One of the clinically most important resistance traits in enterococci is that to vancomycin (Moscoso et al., 2011). Vancomycin-resistant enterococci (VRE) were first reported in hospitals in the 1980s and have since been reported in healthcare settings worldwide. Resistance to vancomycin is typically mediated by acquisition of the vanA or vanB gene cluster (Arthur et al., 1996). VRE are aetiological agents of bacteraemia/septicaemia, surgical wound infections, UTI, and endocarditis (Tannock and Cook, 2002). Infections are associated with excess mortality, prolonged in-hospital stay, and increased treatment costs compared with infections with vancomycin-sensitive strains (Chiang et al., 2017).

Methicillin-resistant *S. aureus* (MRSA) finds its primary ecological niche in the human nose, but is also able to colonize the intestines and perineal region (Acton et al., 2009). MRSA are resistant to all penicillins, cephalosporins, and carbapenems (Raf, 2018). However, staphylococci are mainly transmitted via direct skin contact and indirectly via contaminated surfaces or objects. The gastrointestinal tract is not the natural colonization site for MRSA bacteria, although reported average intestinal carriage rates in healthy individuals and patients were 20 % for *S. aureus* and 9 % for MRSA (approximately half of that for nasal carriage) (Acton et al., 2009).

4.3.1.2 Antimicrobial resistance genes (ARG)

The widespread development of AMR globally has generated concern about the environmental spread of ARGs (Kaushik et al., 2019; Sanderson et al., 2016). ARGs represent a diverse group of genes, being present in a wide variety of microorganisms, both chromosomally and extra-chromosomally. Most clinically relevant ARGs have an environmental reservoir, indicating that their evolutionary role is far more multifaceted than defending against specific antibiotics in the clinical setting (Martinez et al 2008). While developments within genomics and metagenomics enable new opportunities for discovering genes and sequences associated with resistance, the definition of "resistance genes" has become less clear (Martinez et al., 2015). Over recent years, several novel, putative resistance genes have been detected based on sequence homology without functional analysis. Importantly, the occurrence of such sequences in ecosystems does not necessarily pose a risk to human and animal health.

Transferable ARGs located on mobile genetic elements (MGE), such as conjugative transposons, plasmids, and transducable bacteriophages, pose a greater risk of environmental spread through HGT (Colomer-Lluch et al., 2011; Gillings, 2017; Lood et al., 2017; Wellington et al., 2013). Furthermore, the rapid spread of resistance, including MDR, is facilitated by integrons encoding, expressing, and exchanging gene cassettes through a site-specific recombination system (Kaushik et al., 2019). Although HGT seems to be more common between closely related organisms that share the same ecological niches, it has been shown that distantly related bacteria, with different evolutionary and ecological origins, are able to transfer genetic material through conjugation, even in the absence of antibiotics. Massive gene exchanges in completely sequenced genomes were discovered by deviant composition, anomalous phylogenetic distribution, great similarity of genes from distantly related species, and incongruent phylogenetic trees (Doolittle et al., 2003; Jain et al., 2002; Koonin et al., 2001; Kurland et al., 2003; Ochman et al., 2000; Philippe and Douady, 2003).

Generally, exposure of MGE with a broad host range to microorganisms that have a high degree of genomic plasticity represents major concern of inter- and intraspecies exchange.

4.3.2 Mechanisms of antimicrobial resistance (AMR)

4.3.2.1 Resistance to antibiotics

Bacteria can become resistant to antibacterial agents by using one or several of the pathways listed under and illustrated in Figure 4.3.2.1-1: a) Change in the bacterial cell wall permeability; b) Use of efflux pumps; c) Antimicrobial target modification; d) Enzymatic degradation/inactivation of antimicrobials; and e) Alternative pathways.

Multiple mechanisms may be involved in the development of resistance that may be either intrinsic or acquired in nature. The latter could arise as the result of point mutations, e.g.

changing the target site for antimicrobials, or acquisition of resistance genes through HGT by conjugation, transduction, or transformation.

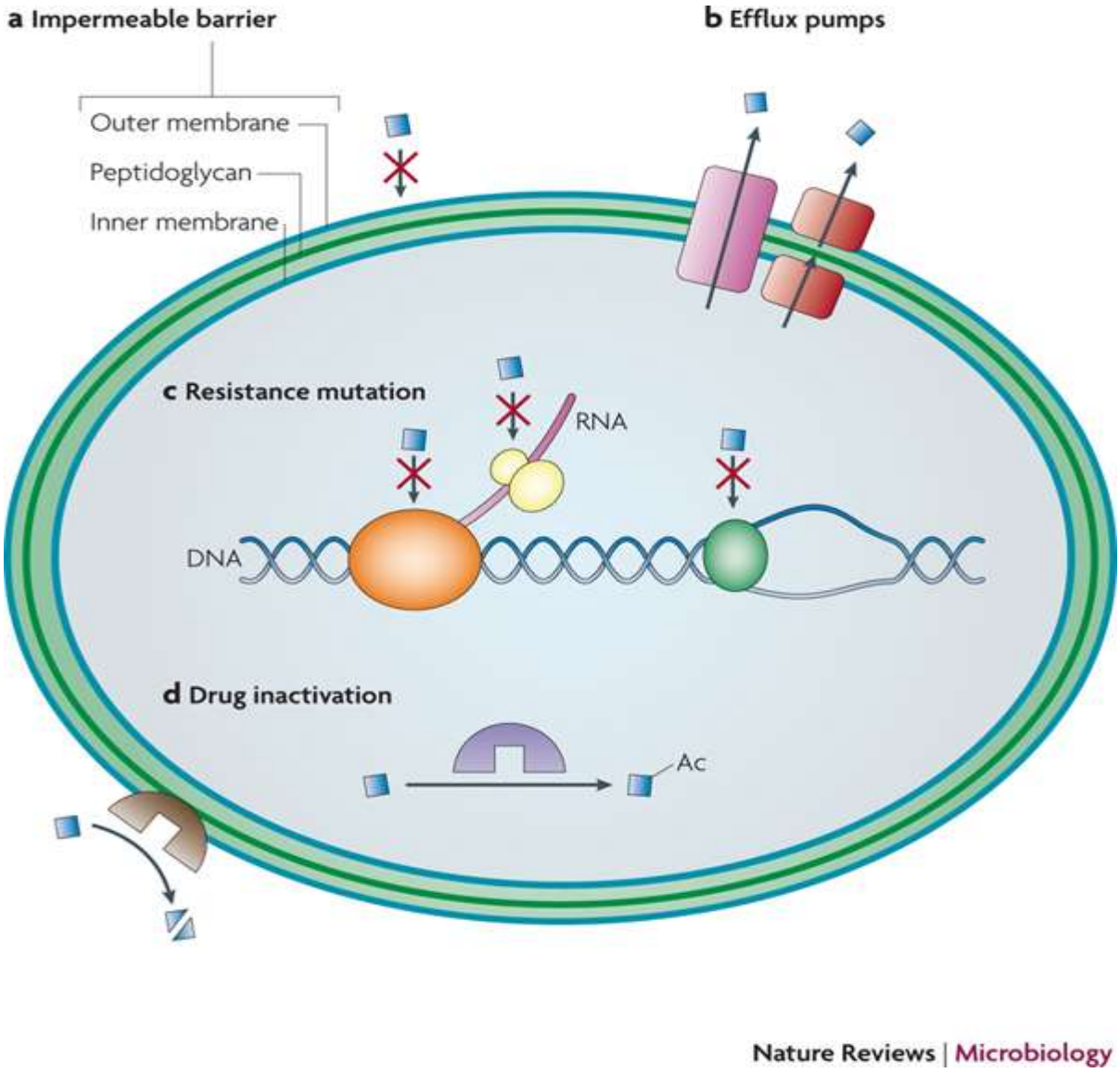

Figure 4.3.2.1-1. Different bacterial resistance mechanisms. Ac: Acetyl group (Allen et al., 2010). Copy allowed from VKM, 2015: Assessment of antimicrobial resistance in the food chains in Norway (Yazdankhah et al., 2015).

4.3.2.2 Resistance to antifungals

Resistance to antifungal treatment occurs for all classes of antifungals. However, it is primarily resistance to azoles and echinocandines that are of concern due to its increasing trend. Resistance to polyenes exists, but is less common (Srinivasan et al., 2014). Fungal species may react to environmental stresses by gene alteration and antifungal treatment can drive the emergence of resistance. Different mechanisms of action for fungal resistance are possible, and can be classified into three categories: 1) altered drug-target or overexpression

of drug target gene; 2) decrease in effective drug concentrations by upregulation of drug transporters and enhanced drug efflux; 3) metabolic bypasses and activation of stress responses. In addition, the formation of biofilms by fungi may prevent antifungal medication reaching its target. Azoles exhibit target-site modification, target abundance, target-site overexpression, drug pump upregulation, non-target effects, and biofilm formation. . Echinocandines exhibit target-site modification, non-target effects, and biofilm formation. Polyenes exhibit target abundance, non-target effects, and biofilm formation (Perlin et al., 2017).

Resistance to antifungals may occur both due to mutations and HGT but the relative importance of this mechanisms are unknown.

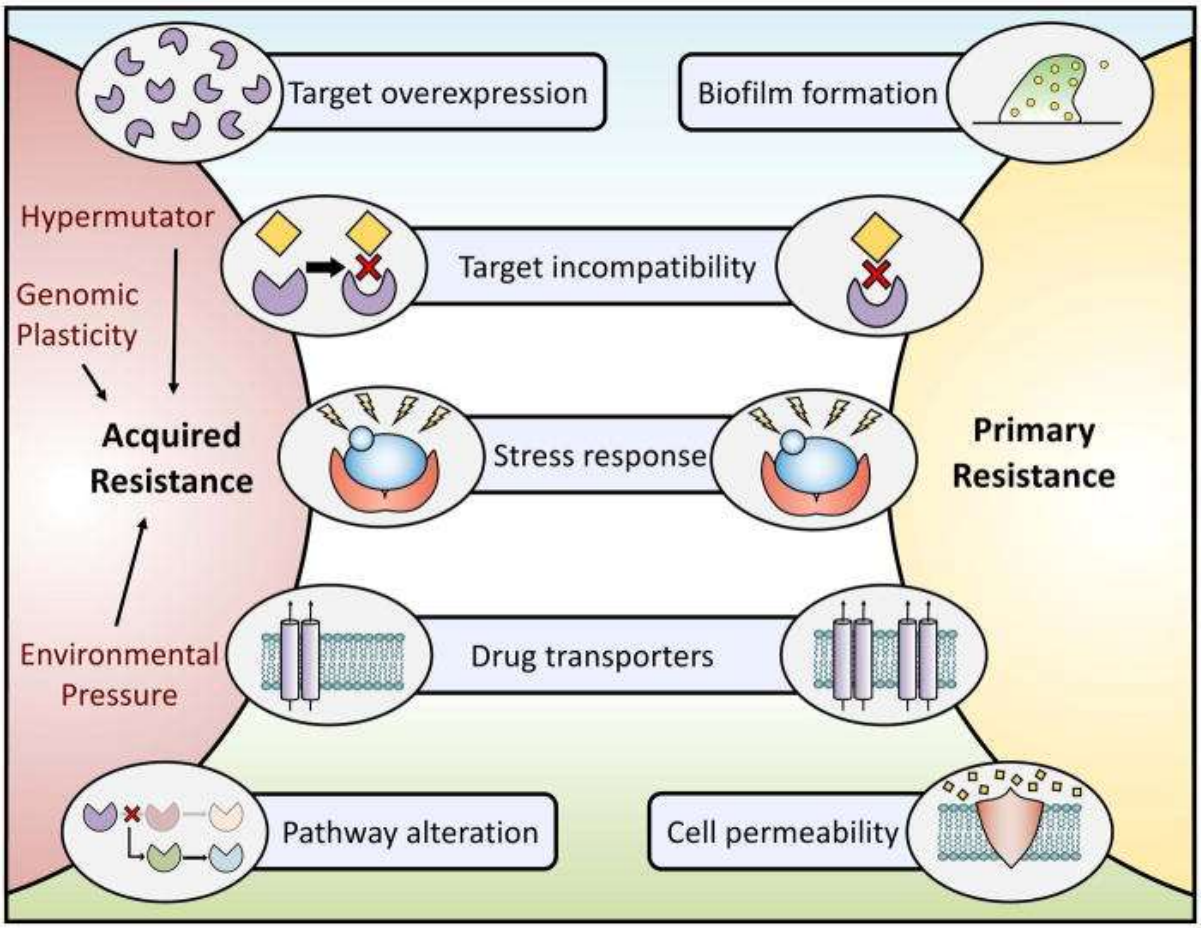

Figure 4.3.2.2-1. Exploring the relationships between and mechanisms governing intrinsic and acquired resistance (Revie et al., 2018)

4.3.2.3 Resistance to potentially toxic metals (PTMs)

In order to avoid cellular toxicity from heavy metals, bacteria have evolved mechanisms of metal tolerance. Both the mechanisms of resistance and tolerance to heavy metals are discussed in the review article of (Seiler and Berendonk, 2012). Resistance mechanisms for PTM may be divided into three groups: a) complex formation; b) detoxification; and c) excretion of toxic ions (Tronsmo et al., 2016). These mechanisms are explained in detail for the different PTM assessed in that opinion.

PTM can contribute to AMR through co-selection based on either of two mechanisms (Singer et al., 2016): (1) co-resistance, where selection for one gene leads to maintenance of another resistance gene that does not necessarily offer a selective advantage to the chemical in question (Johnson et al., 2016); and (2) cross-resistance, whereby one resistance gene can offer protection from multiple toxic chemicals (Curiao et al., 2016). Many examples of cross- and co-resistance between toxic metals and antibiotic resistance have been described in the literature.

Most important are those cases where toxic metal resistance determinants are genetically linked to resistance determinants towards highly important and critically important antibiotics. Emergence of livestock-associated MRSA in pigs is one of the most alarming examples of AMR. The association between resistance to Zn and MRSA of animal origin suggests that the use of Zn as a feed supplement could have contributed to the persistence, amplification, and dissemination of MRSA in pigs, rather than initial development (Wasteson et al., 2017). Mechanisms of resistance against arsenic (As) in bacterial species have been reviewed by Kruger et al., (2013) and (Hobman and Crossman, 2015). The main cross-resistance between As and antimicrobial agents may be activation of efflux pumps (Wasteson et al., 2017).

A study performed by Anssour et al. highlighted bacterial multiple-antibiotic and toxic metal resistance in hospital effluents, which is linked to ciprofloxacin resistance through selective pressure, co-resistance, and cross-resistance (Anssour et al., 2016). This should draw attention to the consequences of exposure of bacteria to fluoroquinolones in general, and ciprofloxacin in particular, through their substantial and/or inappropriate use and their release into hospital effluents.

The concentration necessary to select for or retain resistance genes may be low (Singer et al., 2016), and in some cases microbial selection concentrations sufficiently low as to occur in sewage sludge and soil (<1 mg/L for As and Cu) have been reported (Gullberg et al., 2014).

4.3.2.4 Resistance to biocides (disinfectants)

As biocides (disinfectants) have multiple target sites in a microbial cell, the emergence of general bacterial resistance is unlikely to be caused either by a specific modification of a target site or by a by-pass of a metabolic process. Several mechanisms based on this

principle (mode of action) have been well-described, including changes in cell envelope, alteration in permeability, efflux, and degradation (SCENHR, 2009). Some of the resistance mechanisms are intrinsic (or innate) to the microorganism, whereas others have been acquired (e.g., mutation, acquisition of resistant determinants) through forced mutations or through the acquisition of MGE (Poole, 2002). Innate mechanisms can confer high levels of bacterial resistance («insusceptibility») to biocides (Table 4.3.2.4-1).

Table 4.3.2.4-1. Bacterial mechanisms of resistance to biocides (SCENHR, 2009).

Mechanisms	Nature	Level of susceptibility to other biocides	Cross-resistance
Permeability	intrinsic (acquired)	none	yes
Efflux	intrinsic/acquired	reduced	yes
Degradation	acquired/intrinsic	reduced	no
Mutation (target site)	acquired	reduced	no[2]
Phenotypic change	following exposure	reduced	yes
Induction (stress response)	following exposure	variable	yes

[1]to other biocides - level of susceptibility defined according to the concentration of biocides
[2]not to other biocides, but cross-resistance with specific antibiotics.

4.3.2.5 Resistance to other substances

The mechanisms that bacteria use to handle stress from plant-derived antimicrobial compounds are probably widespread in all bacterial taxa. The activities of phytochemicals and antimicrobial peptides from plants cover a large array of mechanisms and many mechanisms of actions are unknown. Similarly, the resistance mechanisms against the phytochemicals and antimicrobial peptides will be extremely variable. Although the potential of using antimicrobial peptides and phytochemicals from plants in medicine as replacement of traditional antibiotics has been investigated (Barbieri et al., 2017), research on acquired resistance to these compounds is still lacking.

4.4 Sewage treatment

4.4.1 Types of wastewater

The term wastewater (WW) describes all water that has been discharged following human activities. This might be WW from industrial activities, specialized entities like hospitals, households, businesses, and commercial use, runoff, etc.

More precisely, the term urban wastewater, also termed as sewage synonymously, describes WW produced by a group of people in settlements of any size, and contains the effluents from households, small commercial or industrial entities, and, most often, surface runoff. Domestic wastewater is the term used for effluents from households only.

Unfortunately, quite often, the term "wastewater" is used when the intended meaning is urban wastewater (or sewage). In order to avoid misunderstandings, the more precise terms "urban wastewater", and "sewage" should be used.

4.4.2 The purpose of sewage treatment

When natural water bodies are overloaded with nutrients like phosphorous and nitrogen eutrophication may occur, which is accompanied by oxygen depletion and might even result in the total collapse of the ecosystem. In order to avoid such situations, and with the aim of maintaining an environment that is as close as possible to natural conditions, the purpose of sewage treatment is to ensure that the water discharged does not result in any overload or long-term alteration of the receiving ecosystem, or cause the accumulation of toxic substances that might harm the ecosystem. Furthermore, as natural water bodies are often used for drinking water production, substances that are considered as contaminants in drinking water should be removed, or their concentration decreased, by sewage treatment. From a process-engineering perspective, it is easier (and cheaper) to remove substances at high concentrations from a relatively small volume, than when diluted into a much larger volume.

Based on this, it is generally agreed that the degree of WWT should be dependent on the ecological vulnerability of the receiving water bodies and the volumes discharged per unit of time. This is reflected in laws and regulations, such as the European Council Directive concerning urban wastewater treatment (EEC, 1991). [Council of the European Communities (1991). Council directive of 21 May 1991 concerning urban waste water treatment. (91/271/EEC)]

Recent knowledge, mainly gained during the last two decades, demonstrates the necessity of removing contaminants such as pharmaceuticals, personal care products, flame retardants, and plasticizers from sewage effluent. Antimicrobials, as well as ARB and ARG, recently became subjects of focus with increasing awareness of the potential threat resulting from increasing AMR in the environment.

4.4.3 Sewage treatment processes

4.4.3.1 Levels of sewage treatment

The different levels of sewage treatment described below and illustrated in Figure 4.4.3.1-1 also reflect the historic development of WWT technology and legislative requirements. In the early developments of sewage treatment, the focus was on the removal of directly visible objects and solid substances (primary treatment). Then, with increasing awareness of the effect of high organic nutrient loads on the environment, technology for nutrient removal was developed (secondary treatment), and requirements regarding the removal of dissolved and suspended organic compounds, measured as removal of biological and chemical oxygen demand, were increased. Often phosphorous removal is included in secondary treatment, but not necessarily. Further, technologies for phosphorous and nitrogen removal were developed (tertiary treatment), and often incorporated in many WWTP. Today, especially in densely populated areas with high discharge rates, additional treatments of sewage, for the removal of trace contaminants and hygienisation, are requested, with the intention of being realized in some countries during the next twenty years.

Sludge is separated from the sewage during the treatment process, and usually treated separately, and this is discussed in a separate chapter. According to Norwegian legislation, hygienisation of sludge is required (Sundstøl et al., 2009).

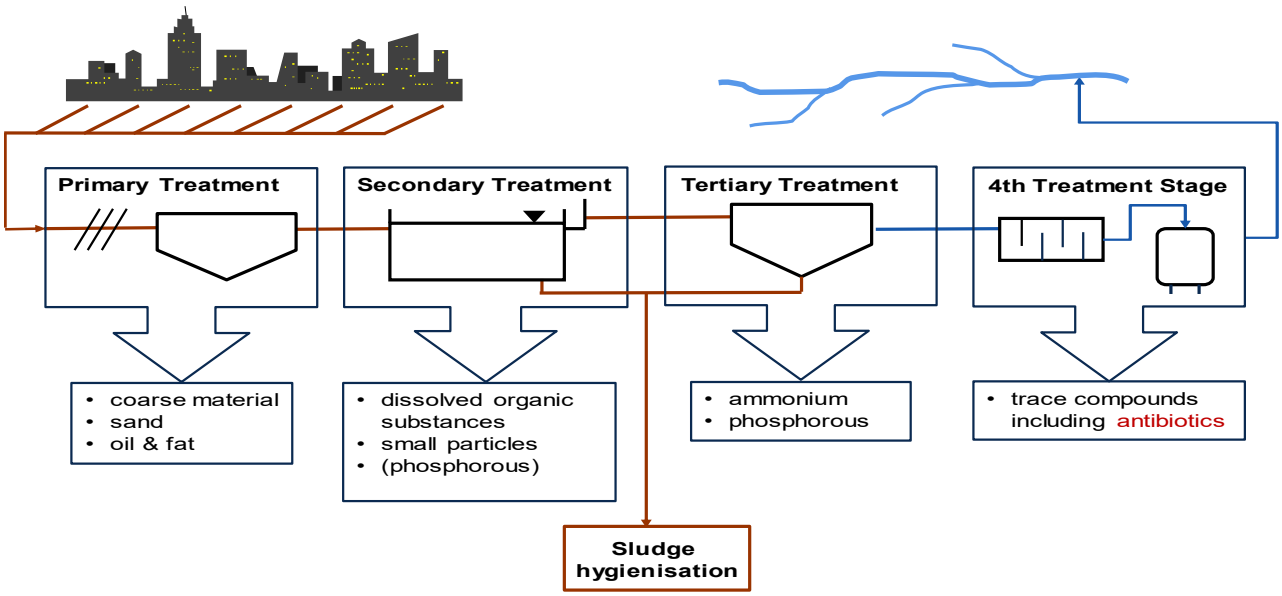

Figure 4.4.3.1-1. Levels of sewage treatment (Illustration by Irene Slavik, with permission).

4.4.3.2 Primary treatment

In primary treatment, which is mechanical, coarse material, such as sand, as well as oils and fats, are removed by sieving, sedimentation, and flotation processes. First, the WW passes

through a screen, where coarse materials, visible to the naked eye, like organic material, paper, plastics, and textiles, are removed and collected in containers. Then the effluent moves to a sand and grease trap. By sedimentation, gravel, sand and other substances of high density are removed in channels or basins, where the flow rate of the water is slow. The sediments are removed mechanically from these channels by scrapers. Grease, which mainly concentrates on the water surface, is removed by skimmers. This flotation can be supported by aeration. Such mechanical treatment is important for the following treatments, as it protects the subsequent treatment stages from plugging and abrasion, and thus increases reliability, ease of operation, and reduces maintenance costs. In the final basin in primary treatment, termed the primary sedimentation basin, the flow rate is decreased further to remove small mineral and organic substances from the WW. The so-called primary sludge collected there is transferred to the sludge-treatment unit.

About 30% of pollutants are removed from WW by primary treatment. The remaining substances, which are predominantly organic, are mainly removed in the biological stage of secondary treatment.

4.4.3.3 Secondary treatment

Secondary WWT is primarily biological treatment. The so-called activated sludge process is carried out in huge basins under aeration. Microorganisms suspended freely and, to a larger extent, sessile on particles or incorporated in flocs, degrade dissolved organic substances. Organic carbon, nitrogen, and phosphorous from dissolved substances are removed and converted to carbon dioxide and biomass, which is incorporated in the sludge. The processes in such a biological stage are the same as during natural self-purification in an aquifer,but are optimized and more rapid than in nature.

Sludge is subsequently separated from the water by sedimentation, in the so-called post-sedimentation basin. The sludge is largely recirculated to the biological process, such that the concentration of biomass in the activated sludge process can be kept higher than without recirculation. Degradation processes are faster, and the volume of the activated sludge basin is lower with recirculation.

An alternative to the activated sludge process is the membrane bioreactor (MBR) process. In this process, instead of recirculating sludge from the sedimentation basin to the biological stage, it is kept in the biological stage and dewatered using membranes. This way the biomass concentration in the membrane bioreactor can be three to four times higher than in an activated sludge process, enabling far smaller volumes of the basins. A further advantage of the MBR process is the production of effluent that is almost free from particulate matter, which also includes bacteria and consequently such bacteria that carry AMR. When the pore size of the membranes, usually described as molecular weight cut-off (MWCO), is small enough, even free DNA, which can include ARG, is effectively removed (Krzeminski et al., 2020; Slipko et al., 2019). However, extended aeration and the pressure needed for the membrane filtration require more energy than the conventional activated sludge process.

In addition to the biological processes, dissolved inorganic phosphorous is often removed chemically. Iron or aluminium salts are added in dissolved form to improve the sedimentation rate of flocs and particles. Under the conditions in the WWT process, they precipitate as hydroxides and phosphates, forming large flocs. The flocs incorporate very small organic and inorganic particles and thereby facilitate their removal in the final sedimentation basin. Removal of phosphorous, as the iron and aluminium salts also precipitate as their respective phosphates, is a beneficial side-effect.

4.4.3.4 Tertiary treatment

For highly loaded WW, standard secondary treatment is insufficient for removing organic compounds and ammonium (i.e., nitrogen) that degrade very slowly. Ammonium especially must be removed in a tertiary treatment step to improve the effluent quality to meet the limits for discharge to the receiving environment. This is also the case for phosphorous, assuming it has not been removed sufficiently in secondary treatment.

Nitrogen, mostly in the form of ammonium ions, must be removed as it causes oxygen consumption in recipient waterbodies, and because ammonia is poisonous to fish. Removal is achieved through nitrification and denitrification processes. Nitrification means the biological oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and further to nitrate (NO_3^-) by autotrophic bacteria under oxygen consumption. In this process, protons (H^+) are also released, which results in a decrease in the pH of the treated effluent. Consequently, sufficient bicarbonate for carbon dioxide formation and aeration, for carbon dioxide discharge to the atmosphere, are necessary. This means that nitrification requires oxygen, bicarbonate, and nitrifying bacteria in the biomass. Because of the slow growth rate of nitrifiers, treatment basins for nitrification must be large enough to guarantee sufficiently high sludge age.

As nitrified waters still contain nitrogen, denitrification processes are necessary to remove the resulting nitrate. Denitrification thus means the microbiological reduction of nitrate to elemental nitrogen. This is done by heterotrophic bacteria, which use nitrate instead of oxygen for the oxidation of organic compounds. Consequently, a complex process management is required to guarantee that none of the organic compounds that are necessary for the anoxic denitrification are removed during the aerobic nitrification. Protons are consumed during denitrification reactions. This results in a release of bicarbonate and, consequently, in an increase in pH. When WWT aims at nitrification and denitrification, separate zones must be available within the basin for nitrogen removal. In summary, denitrification requires organic, biodegradable compounds, nitrate, and heterotrophic bacteria. Dissolved oxygen must not be present.

In order to prevent eutrophication in surface waters, discharge of phosphates from WWTP must be limited. Consequently, removal of phosphorous is often part of tertiary WWT steps. Removal of phosphorous during WWT entails phosphorous precipitation by iron, aluminium, or, rarely, calcium salts. In most cases, trivalent iron salts are used for phosphate precipitation, resulting in insoluble iron phosphates. In this process, iron hydroxide is formed

simultaneously. Due to these competing reactions, overdose with the precipitants is necessary. The precipitates are flocs and suspended compounds that can be separated by sedimentation, together with other sludges.

There is also the possibility of biological phosphorous removal. The advantage of this process is that chemicals are not needed, and less sludge is produced than in chemical precipitation. For biological phosphorous removal, special heterotrophic bacteria are used that are capable of storing polyphosphates intracellularly as a biochemically available energy source. As a result, the phosphorous content of these bacteria increases. If it is possible to separate these bacteria, phosphorous is removed simultaneously. In concludsion, biological phosphorous removal requires the simultaneous fulfilment of the following criteria: (i) in an anaerobic reactor, dissolved organic and biodegradable compounds must be present, but no nitrate and oxygen. (ii) Subsequently, an aerobic or anoxic part of the reactor is necessary. (iii) Biomass has to be adapted. (iv) The phosphorous-rich sludge must be separated from an aerobic partial flow as excess sludge.

4.4.3.5 Advanced (quaternary) treatment

Increasing awareness about substances generally termed "trace contaminants", such as hormones, pharmaceuticals, pesticides, fungicides, and chemicals of industrial origin, has stimulated political discussions, research, and further economic evaluations for developing a fourth WWT stage that would remove such substances effectively and cheaply. The concentrations of these substances are very low and in the order of magnitude of µg/L or even ng/L in aquifers, and often even in WW. Most of these substances are persistent in the environment and are of ecotoxicological concern. As WW is a point source for their dissemination into the environment, further (quaternary) WWT is generally considered necessary in very many cases in order to guarantee a "good ecological status" of the aquatic environment, as is requested according to the Water Framework Directive (Chave, 2001). Such fourth WWT stages are currently being implemented in large WWTP in Switzerland and Germany, as well as in small plants discharging to sensitive areas.

Trace contaminants are mainly introduced into the sewage system from diffuse sources. Their concentrations increase during transport to the WWTP, where they are at their maximum. As it requires less technical effort to remove substances at high concentrations than at low concentrations, it is logical to introduce treatment for removal of trace substances at the WWTP, and this is also true from an economic perspective.

One treatment method under close consideration is the application of powdered activated carbon (PAC) or granular activated carbon (GAC). Both preferably remove hydrophobic trace contaminants, by adsorption to the very high pore surface area in the order of magnitude of 1,000 m^2/g. After the adsorption has taken place and the adsorptive capacity is exhausted, GAC is regenerated, while PAC is removed from the water by sedimentation or filtration. The reuse of activated carbon that has previously been applied in drinking water treatment can be considered, as this would be economically beneficial for both.

Ozonation is another treatment process that has been frequently discussed and considered. Ozone, which is produced from pure oxygen, usually using corona discharge, is a very reactive oxidant and oxidizes all kinds of organic substances. The reaction products are usually easily biodegradable and can be removed by biofiltration or will be degraded downstream from the WWTP. However, as WW is a very complex mixture of a variety of different substances, the types and concentrations of the reaction products cannot be easily predicted.

4.4.4 Treatment of sewage sludge

From all processes and in all stages of WWT, considerable amounts of sludge are produced. These sludges include all compounds that have been removed from the WW during treatment, and that have not been degraded or mineralized during the biological processes that are part of the treatment: biomass, nutrients, heavy metals, manmade organic compounds, biohazardous material etc. The main objective of sludge treatment is, therefore, to convert the sludges into a state in which the components can be used in agriculture, and residues can be disposed of in a controlled landfill or or utilized as energy source in waste incineration plants or industrial furnaces. The treated sludge must thus be harmless to the environment, and suitable for final disposal or use.

For sludge treatment, a variety of mechanical, physical, biological, and thermal processes are available. For mechanical removal of sludge water as far as possible, and thus to reduce sludge volume, thickening is applied. By thickening, particulate material is concentrated by gravitation (i.e., sedimentation). After sedimentation, further removal of water from the sludge can be achieved. In so-called conditioning, flocculants are added to cause particle aggregation. Finally, decanter centrifuges or filter presses are used to separate water from the flocs. During these treatment steps, dissolved compounds remain unaffected in the water phase. These must be disposed of with the sludge water, or the sludge water must be treated further. In several WWTPs, conditioning also involves liming with CaO. This increases the pH of the sludge, which makes phosphates more plant-available, and contributes to hygienisation.

After these treatment steps, only thermal processes can be used to dry the sludge solids further through evaporation. A wide range of different designs of drying plants exists, and different kinds of heating can be used (e.g., steam, hot exhaust fumes, or carrier oil). As sludge might contain high concentrations of organic substances, this can result in considerable production of odorous gases. Sludge stabilization ensures that easily decomposed substances are mineralized.

For sewage sludge, even dried sludge, to be used in agriculture, hygienisation is required. This is a thermal pasteurization process at 70 °C. During warming up, the particulate organic material is partly transferred into a dissolved state, and might then serve as nutrient. Due to the heating, most bacteria are killed. However, pathogens might proliferate during re-cooling. To avoid regrowth of pathogens, the hygienisation process is carried out in a

controlled manner in digestion towers, where only wanted (harmless) bacteria can grow, and the dissolved compounds are degraded. The growth of pathogens is prevented by strong competition with harmless bacteria and the sludge remains hygienised. In Norway, hygienisation and stabilization of sludge is required by law (Lovdata, 2003).

The energy content of sewage sludge can be used if the sludge is combusted. Due to the resulting flue gases, flue gas cleaning is required.

Combustion is performed either in industrial furnaces or in fluidized bed furnaces (co-incinceration combustion). In industrial furnaces of cement plants, the sludge is used as a substitute for fossil fuels and the ash is incorporated in the product. However, the utilization of nutrients such as phosphorous is impossible then.

Besides co-incineration combustion, mono-incineration combustion plants are used which solely combust sewage sludge. Phosphorous can be recovered from the ash from mono-incineration plants. A report by the German Environmental Agency (UBA) concludes that about 55 % of the German agricultural use of phosphorous could potentially be recovered if all sewage sludge was incinerated in mono-incineration combustion plants (Umweltbundesamt, 2013).

The state of sewage sludge treatment in Norway is summarized in chapter 4.4.6.3.

4.4.5 Treatment of hospital wastewater

A summary of hospital effluent treatment processes is given by Asfaw, and includes activated sludge treatment, oxidation ditches, MBR, convectional activated sludge, integrated anaerobic-aerobic fixed-film reactors, and WW stabilization ponds (Asfaw, 2018). The author points out the different removal efficiencies with respect to pathogens. Activated sludge treatment and oxidation ditches were assessed as being less effective in eliminating bacteria and parasites from hospital effluent, whereas MBR may play a key role in hospital WWT because of high removal of bacteria. Furthermore, it is stated that waste stabilization ponds can attain a 99.9 % faecal coliform reduction, and 100 % removal of helminths, thus facilitating the recovery of the WW for agriculture in both restricted and unrestricted irrigation. However, it is also pointed out that certain resistant bacteria may pass through the WWTP.

Paulus et al. investigated on-site hospital WWT in a Dutch city, including MBR, ozonation, GAC, and UV-treatment (Paulus et al., 2019). Advanced on-site treatment removed between 0.5 and 3.6-fold more genes than conventional biological urban WWT (activated sludge). MBR treatment was most efficient in reducing ARGs and ozonation in reducing ARB. These results indicate a positive effect of on-site treatment of hospital WW on the communal sewage system.

Dires et al. studied artificial wetlands in Ethiopia to evaluate their effectiveness in the removal of ARB from hospital WW (Dires et al., 2018). A significant number of ARB were

removed in vegetated broken brick and gravel bed wetlands, whereas removal in non-vegetated gravel bed wetlands was lower. This indicates the positive use of plants in ARB removal from wastewater. Timraz et al. showed that activated sludge processes operating on-site at two hospitals in Saudi Arabia effectively removed ARB from most effluent samples (Timraz et al., 2017). However, ARG remained detectable in the treated effluent. Consequently, ARG can become a potential source of HGT in the receiving municipal WWTP.

Szekeres et al. report on a hospital in Romania where an activated sludge and chlorine disinfection process were applied before the release of WW to the municipal sewage system (Szekeres et al., 2017). This conventional WWT showed moderate removal effectivity for the pollutants studied, with a 55 to 81 % decrease in antibiotic concentrations, 1 to 3 orders of magnitude lower relative abundance of ARG, but with a slight increase in some potentially pathogenic bacteria.

Lucas et al. report on the treatment of WW from a veterinary hospital in a fungal bioreactor (Lucas et al., 2016). In this study it was shown that 77 % of antimicrobials were removed, and the fungal treatment was also effective at removing ARG.

The recycling of hospital effluents in plants consisting of equalizer tanks, aeration tanks, sedimentation tanks, sand filters, carbon filters and a final discharge to lagoons for natural sedimentation was investigated by (Kalaiselvi et al., 2016). The authors observed a decrease in microbial load, but some pathogens evaded removal due to inadequate filtration and survived in the recycled water.

Manonmani and Catharin studied a hospital effluent treatment plant in India, consisting of an equalisation tank, an aeration tank, a settling tank, a chlorination tank, a polishing tank, and sludge drying beds (Manonmani and Catharin, 2015). They observed a substantial reduction (> 2 log) in the settling tank and noticed that most of the bacteria remained tightly attached to the solid particles. The latter were removed by aeration and clarification or settling after flocculation. The treated effluent still contained sizeable bacterial loads although disinfection procedures, like chlorination, were followed. Bacteria in sludge drying beds were exceedingly robust and higher concentrations of chlorine were required for decontamination, and other disinfection procedures (such as UV radiation, ozonation, and sunlight disinfection) were recommended for their reduction.

According to Liu et al., MBR are used at a large scale in China to treat hospital WW (Liu et al., 2010). Pauwels and Verstraete report on MBR treatment at the Kinki University Nara Hospital, Japan, that ensures a 7-log reduction in pathogens (Pauwels and Verstraete, 2006). These authors furthermore recommend activated carbon, ozonation, and UV photolysis as suitable post-treatment technologies to remove hospital-related pollutants. They underline the by-product issue of ozonation and consider reverse osmosis as impossible to apply because of the necessary pre-treatment.

Chitnis et al. and Ravikant et al. studied the efficiency of an Indian hospital WWTP, with terminal chlorination to produce a water usable for irrigation and sanitary clearing (Chitnis et

al., 2004, Ravikant et al., 2002). The treatment aims at addressing the high-load of MDR bacteria in particular, and includes bar screens to remove coarse suspended solids, an oil and grease separator, an aeration tank where aerobic bacteria oxidize the suspended and dissolved organic matter, a clarifier tank to separate suspended biological material (part of the sludge is returned to the aeration tank to provide biomass for the treatment, excess is discharged to a sludge drying bed), and a sand filter to remove the fine suspended matter. The final chlorination step is to inactivate the remaining microbial population.

Tsai et al. focus on the appropriate treatment and disposal of hospital sludge that contains pathogenic species removed from hospital WW (Tsai et al., 1998). The methods used to inactivate microorganisms in the waste sludge include aerobic digestion, anaerobic digestion, composting, air drying, and lime stabilisation.

4.4.6 Requirements and state of sewage treatment in Norway

4.4.6.1 Requirements for urban waste water treatment (UWWT) in Norway

The Norwegian Pollution Regulation for discharge of wastewater (Forurensningsforskriften, Lovdata 2004) includes both individual systems in scattered settlements (up to 50 pu), from rural agglomerations and for urban agglomerations. Largely standardized, minimum treatment requirements are set out in the Regulation. The authorities can set stricter standards to provide adequate security for surface water bodies.

The regulations of discharge of wastewater from urban agglomerations are based on the Urban Waste Water Treatment Directive (UWWTD), which is a part of the Norwegian Pollution Regulation for discharge of wastewater. The UWWTD distinguishes between treatment of discharges of WW into receiving waters which are considered as sensitive, normal or less sensitive area. The most stringent requirements to WWT is to be applied for discharges into sensitive areas and less stringent requirements can be applied in the less sensitive areas.

In Norway, the same differentiation in requirements for receiving waters are used for regulation of discharges from rural agglomeration, even though the requirements to WWT are less stringent.

Generally, definitions are as follows:

Sensitive areas: natural lakes and other surface waters, river mouths or deltas, fjords and other surface waters that are eutrophic, or could become eutrophic if not protected.

Less-sensitive areas: A marine water body or area can be identified as a less sensitive area if the discharge of waste water does not adversely affect the environment as a result of morphology, hydrology or specific hydraulic conditions which exist in that area.

Normal areas: Freshwater bodies which are not identified as sensitive

Based on these criteria, the following areas in Norway have been identified as sensitive, normal, and less sensitive as described below.

Sensitive: The coastline from the Swedish border to Lindesnes, including the catchment areas and the area at the Grimstadfjord (Nordåsvannet, Grimstadfjorden, Mathopen and Dolviken).

Normal: All freshwater bodies in Norway that are not classified as sensitive.

Less sensitive: All coastlines and river mouths from Lindesnes to the border at Jakobselv that are not classified as sensitive.

The sensitive areas, catchment areas contributing to the sensitive areas, and normal areas, are shown in Figure 4.4.6.1-1.

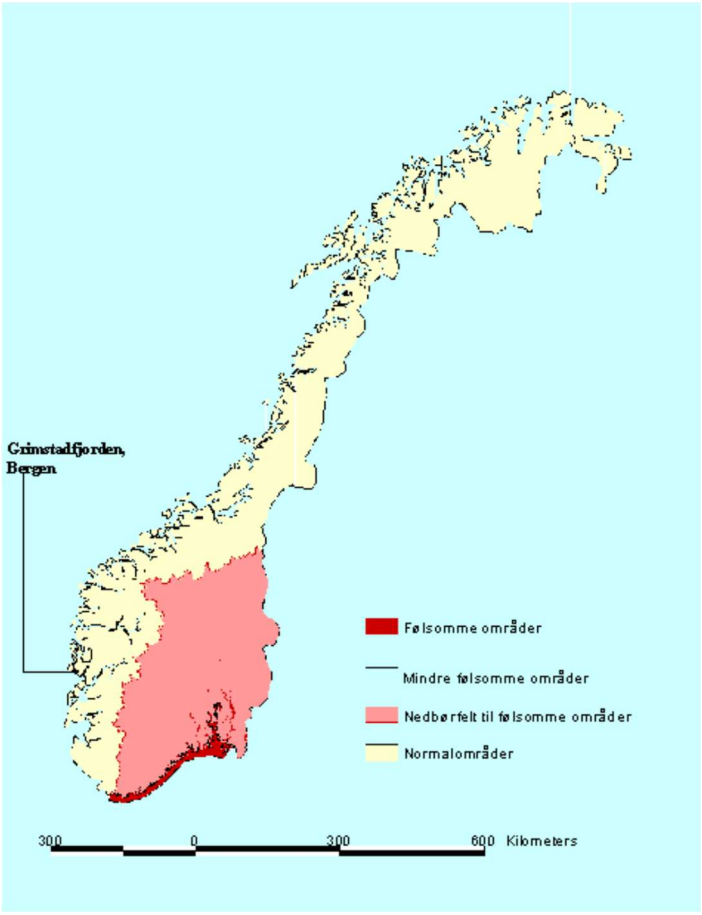

Figure 4.4.6.1-1. Description of areas in Norway as "sensitive", "less sensitive", and "normal". In addition "catchment areas to sensitive areas" (Lovdata, 2004).

According to the UWWTD, primary treatment requires a WWT technology that reduces BOD5 by 20 % and suspended solids (SS) by 50 %. Secondary treatment involves treatment processes that reduces the biochemical oxygen demand (BOD_5) by a minimum of 70 % and chemical oxygen demand (KOF) by a minimum of 75 %.

In the UWWTD, secondary treatment reflects the treatment standard for discharges into receiving waters considered as normal. For discharges into less sensitive area, primary treatment may be adequate, and for discharges into sensitive area, tertiary treatment as minimum 70 % nitrogen removal or 80 % phosphorous removal is a demand as well. In Norwegian regulation, phosphorous removal by 90 % is required as a minimum.

For discharges from rural agglomerations into receiving waters considered as sensitive or normal, the requirements for WWT are 98% removal of
phosphorous. When discharging is to a less-sensitive area, the requirements are 20 % removal of- SS.

4.4.6.2 State of sewage treatment in Norway

Main treatment processes applied in Norwegian sewage treatment plants in both rural and urban settlements are presented in table 4.4.6.2-1.

In Norway, the largest amount of treatment plants is based on mainly mechanical treatment processes. This reflect the high degree of rural settlements in Norway, and specially by the long coastline from Lindesnes to the border at Jakobselv, with discharges of WW to less sensitive areas. In this area, it is still some small plants with untreated direct discharge of WW.

Based on the requirements in the Pollution Regulation (Lovdata, 2004), it is largely WWTP that discharges to the catchment of the coastline from the Swedish border to Lindesnes, see figure 4.4.6.1-1, that use further treatment (i.e., at least secondary treatment) as chemical, chemical and biological or biological treatment processes, see table 4.4.6.2-1. This is also the part of Norway where about half the population lives.

In this region, a high degree of phosphorous removal is established. A few urban agglomerations have tertiary treatment as nitrogen removal, these are: Nordre Follo, Oslo, Jessheim and Lillehammer.

Table 4.4.6.2-1. Main treatment processes applied in Norwegian sewage treatment plants 2018, Statistisk Sentralbyrå (2020)

Process	Number of plants	Number of plants (%)	Connection (1000 people)	Connection (%)
Untreated direct discharge	374	13.8	103.1	2.2
Mechanical	1412	52.0	881.8	19.1
Biological	91	3.3	398.4	8.6
Other processes	211	7.8	275.8	6.0
Chemical	225	8.3	1 129.6	24.3
Chemical and Biological	404	14.9	1 833.7	39.8
Total	2 717	100	4 612.5	100

4.4.6.3 State of sewage sludge treatment in Norway

In Norway, the vast majority of sludge is treated employing either hygienisation pre-treatments, biological treatments with temperatures that kill intestinal bacteria and parasites, and/or thermal drying. A few small WWTPs do however exist where untreated sludge is only dewatered mechanically and then treated by composting, lime amendments and/or long-term storage (2-3 years). While lime may kill bacteria and parasites, low temperature composting and long-term storage are not considered adequate for fulfilling the requirements of the Norwegian fertiliser regulation. Due to long-term outdoor storage, such sludge also teems with weeds. It is not used as soil amendments in agriculture or landscaping.

The following nine methods commonly used in Norway are illustrated, including dewatering, in Figure 4.4.6.3-1. In more detail, the methods are described in VKM (2009).

- Thermophilic aerobic digestion
- Thermophilic aerobic pre-treatment + mesophilic anaerobic digestion (dual digestion)
- Pre-pasteurisation + mesophilic anaerobic digestion
- Thermal hydrolysis + mesophilic anaerobic digestion
- Mesophilic anaerobic digestion + thermal drying
- Thermophilic anaerobic digestion

• Composting (windrow or in-vessel)

• Lime treatment (addition of quicklime to dewatered sludge)

• Long-term (min. 3 years) storage of dewatered sludge

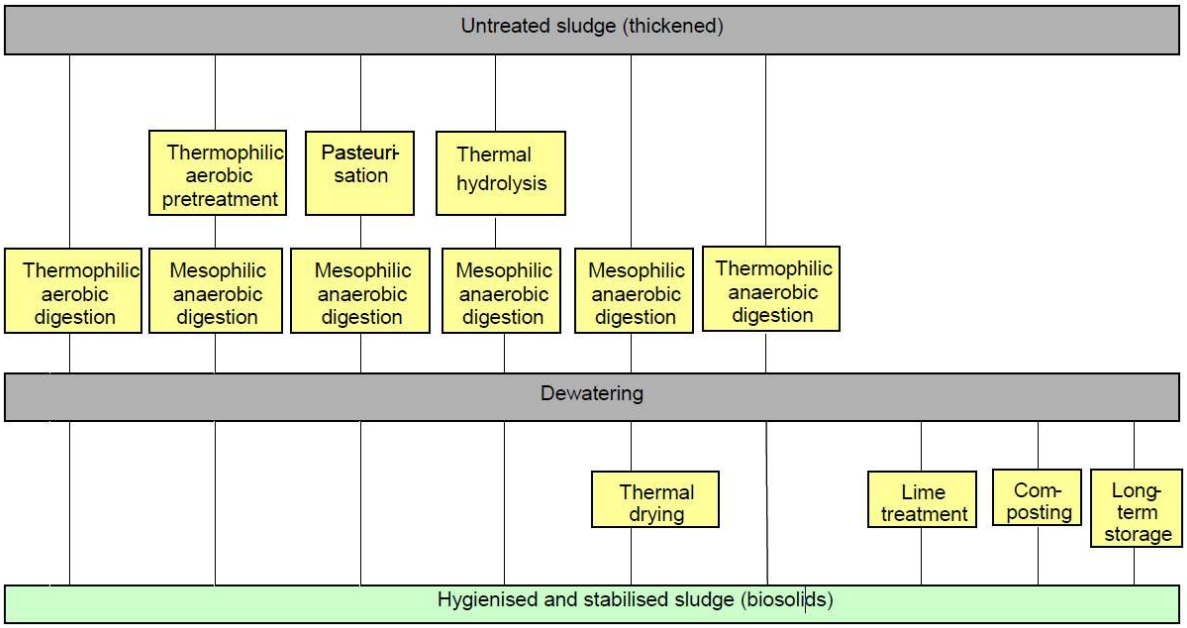

Figure 4.4.6.3-1. Processes applied for stabilization and dewatering of sewage sludge in Norway (VKM, 2009).

5 Occurrence and fate of ARB and ARG in WWTPs

5.1 General remarks

This chapter presents a range of studies describing different antimicrobial resistant bacterial populations, bacterial species and bacterial genes arriving at the WWTPs, their fate through the plants and their release via either the effluent water or sludge fractions. Special emphasis is given to specific ARBs or ARGs regarded as emerging threats, and one paragraph, as requested from NFSA and NEA, focuses specifically on ARB and ARG in hospital wastewater. Some information is given on antifungal, biocide and PTM resistance in WW, effluent and sludge, although a lot less knowledge about resistance to these antimicrobials is available.

At the end of chapter 5, a summary of the findings regarded as most important and relevant for this opinion is highlighted.

5.2 Occurrence and fate of ARB

5.2.1 ARB in general

The intestinal microbiota can be regarded as an ecosystem that is characterised by several dominant groups of bacteria that are common in a given population, while, at the species level, there is individual variation in terms of composition (Browne et al., 2017). Intestinal microbiota also contains yeasts, moulds, protozoa, and viruses (Belizario and Napolitano, 2015). The specific composition and total number of intestinal microorganisms are primarily dependent on factors such as diet, medication, age and environment. It mainly consists of obligate anaerobic bacteria. However, a major shift already takes place in the WW pipe and in the WWTP most bacteria are facultative anaerobes (Bengtsson-Palme et al., 2016). The occurrence of resistance amongst these bacteria in WW systems mirrors the carriage rate in society (Kwak et al., 2015; Reinthaler et al., 2013; Zarfel et al., 2013). In a Norwegian study comparing the level of antimicrobial-resistant *E. coli* in sewage from a hospital, a residential area, and the influent water of the WWTP, it was found that the levels of resistant *E. coli* bacteria were reduced during transport through the sewage pipelines and holding tanks towards the WWTP plant (Paulus et al., 2019). Apart from antimicrobial agent use, environmental temperature and WWTP size are important factors related to persistence of resistance and further dissemination in the environment (Parnanen et al., 2019). Although concentrations of tetracyclines and fluoroquinolones in WW and sludge may exceed minimum selective concentrations (MSC) (Kraupner et al., 2018; Lundstrom et al., 2016; Sundst et al., 2009), the number of generations that enteric bacteria undergo in a WWTP are

limited (Flach et al., 2018). However, through the WWT process, ARG were not reduced to the same extent as faecal bacteria (Bengtsson-Palme, 2016; Sharma et al., 2016). Bengtsson-Palme suggested that this was due to ARGs having several hosts within the WWTP and that taxonomic shifts within the WWTP affected ARG occurrence more than potential selection processes (Bengtsson-Palme, 2016). In a Portuguese study, temperature and organic load (measured as COD) in the WWTPs had the most effect on the bacterial community structure. Novo et al. and Bengtsson-Palme stressed the need for comprehensive analyses of resistant/non-resistant strains within relevant species within WWTPs (Novo et al., 2013, Bengtsson-Palme, 2016). Hence, the focus in chapter 6.2 is on studies using culture-dependent methods for resistant bacteria of particular species.

Problematic clinic-related AMR concerns a limited number of different bacterial genera, in particular the ESKAPE (*Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and *Escherichia*) pathogens (Nesme and Simonet, 2015). As faecal-oral transmission is the dominant route with respect to the terms of reference, the main objective in this assessment has been considering the treatment efficiency for resistant enteric bacteria. Therefore, coliform bacteria/Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, have been used as indicators for resistant Gram-negative bacteria and intestinal enterococci for Gram-positive bacteria. Additionally, one sub-section focuses on emerging risks, such as ESBL-producing Enterobacteriaceae (ESBL-E), including carbapenamase-producers, due to the importance of third- and fourth-generation cephalosporins and the increasing carriage rate of resistance plasmids (see further below) (Canton and Coque, 2006; Karanika et al., 2016; Stewardson et al., 2016; Woerther et al., 2013).

Bacterial concentrations of the chosen indicators are typically around 10^5 CFU ml^{-1} for coliforms and *E. coli*, and 10^4 CFU ml^{-1} for intestinal enterococci (Bengtsson-Palme, 2016; Ottoson et al., 2006a; Schwermer et al., 2018; Stenström, 1986). During treatment, sedimentation processes will enrich bacteria that end up in the sludge. Bacterial concentrations in this latter fraction can be up to 10,000 times higher than in untreated WW on a weight basis, but are normally 100 – 1,000 times higher in numbers (Stenström, 1986). Studies on reductions in Swedish and Norwegian WWTP have considered comparable system solutions, antibiotic usage, and climatic factors. Removals during secondary treatment (activated sludge) were in the range of 80 – 99.9 % (Ottoson, Hansen, Westrell, et al., 2006; Stenström, 1986, Flach et al, 2018) (Table 5.2.4-1). Filtration processes enhance removal and may be as high as 5 $\log_{10}$ removal (99.999 %), as was shown for *E. coli* in a pilot MBR (Ottoson, Hansen, Bjorlenius, Norder, & Stenstrom, 2006) or after ultra- and nanofiltration (Schwermer et al., 2018). Direct coagulation over a rapid sand filter for extended phosphorous removal, as deployed in Henriksdal WWTP in Stockholm, resulted in a 3.2 ± 0.8 $\log_{10}$ removal for *E. coli* as well as intestinal enterococci (Ottoson, Hansen, Bjorlenius, et al., 2006; Kwak et al., 2015) (Table 5.2.4-1).

WWT with ozone- and UV-disinfection (or photocatalytic oxidation) are widely recognised as increasing pathogen inactivation, as well as degradation of pharmaceuticals, including

antibiotics (Wang and Zhuan, 2020). Hess et al. reported 0.2 – 5 $\log_{10}$ reduction of enterococci after treatment with ozone (4 mg/L, 5 min in laboratory studies), but with differences between species and related to antibiotic susceptibility (Hess et al., 2016). Inactivation of resistant *E. faecium* strains was higher than inactivation of sensitive strains. For *E. coli*, the corresponding figures were 0.3 – 4.1 $\log_{10}$ removal overall (mean 2.8) and with a relative increase in the proportion with AMP resistance, and a decrease in the proportion with resistance to CIP and ESBL. In a pilot-scale reactor, (Luddeke et al., 2015) reported around one $\log_{10}$ lower numbers of *E. coli* and enterococci after ozonation of primary-treated WW than after tertiary treatment with flocculation and filtration (Luddeke et al., 2015). Although the percentage of ARB increased during ozonation, the concentrations of antibiotic-resistant *E. coli* and enterococci in the effluents of the pilot plant were lower than their respective concentrations in the effluent of the tertiary-treated WW (Luddeke et al., 2015).

A meta-analysis in which 303 data points on the inactivation of *E. coli* from UV treatment in water were collected[2] showed no significant difference between the inactivation of resistant *E. coli* compared to sensitive *E. coli* at different UV levels. In a pilot study on WW, inactivation of enterococci and *Enterobacteriaceae* was 99.97 % and 98.61 %, respectively, but no determination of change ARB was reported. However, based on ARG removal compared to removal of total 16S rDNA, no selection of ARB occurred during the treatment process (Sousa et al., 2017). In contrast, Meckes reported higher percentages of coliforms resistant to tetracycline (TET) and chloramphenicol (CMP) after UV-treatment, to which an R-factor of mediated resistance was assigned (Meckes, 1982). Coliform inactivation was around 3 $\log_{10}$ for resistant isolates compared to 3.4 $\log_{10}$ for sensitive isolates (Meckes, 1982).

In a review by Li et al, UV treatment was described as decreasing the relative resistance of ARG compared to total 16S rDNA counts in all observations (n = 12) whereas ozone led to a relative decrease in 13 and an increase in 6 (Li et al., 2019). When an increase in relative resistance was measured, it mainly involved tet-genes (Li et al., 2019).

5.2.2 Gram-negative bacteria

Of the thirteen studies fulfilling the inclusion criteria for this assessment, eleven (Flach et al., 2018; Guardabassi et al., 2002; Korzeniewska and Harnisz, 2013; Kotlarska et al., 2015; Luczkiewicz et al., 2010; Osinska et al., 2017; Reinthaler et al., 2003; Scheurer et al., 2015; Schwermer et al., 2018; Turolla et al., 2018; Verburg, 2019) did not find any significant selection of resistant coliform bacteria (most often *E. coli*) against the studied antimicrobials during WWT (Table 5.2.4-1). However, Ferreira da Silva et al. reported a significantly higher proportion of *Escherichia* spp. isolates being resistant to ciprofloxacin and cephalotin in the effluent of a Portuguese WWTP than in the influent (Ferreira da Silva et al., 2007). Mokracka

[2] The study included inactivation in different types of water, mainly drinking water and saline solution

et al. also detected significantly higher frequencies of class 1 and class 2 integron-carrying *E. coli* resistant to cephalosporins (cefazolin, ceftazidime, cefoperazone, and cephalothin) in effluent than in influent of a WWTP (Mokracka et al., 2012). Furthermore, significantly higher frequencies of resistance towards piperacillin/tazobactam were reported, whereas resistance to tetracycline, norfloxacin and trimethroprim was significantly lower in the effluent. The frequency of MDR did not show a difference between stages (influent WW, aeration tank, and effluent) of WWT (Mokracka et al., 2012) (Table 5.2.4-1).

Three of the 13 studies indicated a slight increase in resistance frequencies (Korzeniewska et al., 2013; Kotlarska et al., 2015; Luczkiewicz et al., 2010). However, as only a few isolates were analysed, the results were not significant. The most comprehensive AMR screening from a WWTP included 4 028 *E. coli* isolates, and the results provided no support for selection of AMR when data from multiple samplings were aggregated. In addition, there was no increase in the multiple antibiotic resistance (MAR) index (Flach et al., 2018). The WWTP studied (Ryaverket, Gothenburg, Sweden) serves a rather large population and WWTP antibiotic concentrations should therefore be representative for municipal treatment plants in regions/countries with similar antibiotic consumption patterns and water use per capita (e.g., Norway).

Paulshus et al. compared the level of antibiotic-resistant *E. coli* in sewage from a national reference hospital (Rikshospitalet, Oslo) with an urban residential area (Bærum) in the same city area (Paulshus et al., 2019a). The levels of antibiotic resistance in *E. coli* were higher in the hospital sewage as expected, but although the levels of resistance of *E. coli* in the sewage from the residential area were lower, they were similar. In addition, in the sewage from the residential area secondary fermentation-like situations could be developed in the collection tanks. An interesting trend observed in the same study was a reduction in the level of antibiotic resistance in the *E. coli* in the sewage during pipe transport to the WWTP.

5.2.3 Gram-positive bacteria

In a German study, concentrations of staphylococci in sewage were lower than those of *E. coli* and enterococci, and decreased the most during WWT. After secondary settling, numbers were generally lower than 10 CFU/100 ml (Hess et al., 2016) and MRSA has not been considered further for these transmission routes. However, an increase in vancomycin use has led to the emergence of glycopeptide-resistant *S. aureus* (VRSA). One resistance type was due to acquisition of the vanA operon from *Enterococcus faecalis,* resulting in high-level resistance (Perichon, 2009). Hence, there is an indirect risk with VRE in relation to MRSA that may need consideration. Community carriage rates of VRE in the Netherlands have been reported to be 2 % (4/200) (Endtz et al., 1997).

In Swedish and Norwegian hospitals, vancomycin is only used for treatment of serious infections caused by MDR Gram-positive bacteria, such as MRSA, ampicillin-resistant enterococci (ARE), or enterocolitis caused by *Clostridium difficile* (Raf, 2018). Nevertheless, VRE were commonly found in Swedish WWTPs (Iversen et al., 2002). Furthermore, several

studies report the potential adaptation of clinical strains in WWTPs based on strain similarity (Gouliouris et al., 2019; Oravcova et al., 2017) and the identification of clusters with identical or similar isolates from different WWTPs, including hospital WW, collected at different occasions (Iversen et al., 2002).

We identified seven studies that presented figures on the removal efficiency of resistant enterococci during WWT, of which four reported no significant selection (D'Costa et al., 2006; Scheurer et al., 2015; Varela et al., 2013; Xu et al., 2007) and three (Ferreira da Silva et al., 2006; Gouliouris et al., 2019; Iversen et al., 2002) reported selection for resistance towards ten antibiotics[3] from sludge and wastewater before and after treatment in 14 Portuguese WWTPs. No significant differences were found in the resistance towards any of the antimicrobials regarding the origin of the sample (inflow, sludge, and effluent). WWT resulted in a decrease in enterococci of between 0.5 and 4 $\log_{10}$ (D'Costa et al., 2006). In another Portuguese study,(Varela et al. reported two $\log_{10}$ reduction through the WWTP, with no significant difference between ratios of enterococci enumerated on agar with 1 mg/L ciprofloxacin or 16 mg/L vancomycin to unsupplemented medium from raw and treated WW respectively (Varela et al., 2013). In a study by Scheurer (2015) few isolates were included (32 influent and 18 effluent) and no conclusions could be drawn regarding whether sand filtration of combined sewer overflows resulted in any selection for resistant strains (Scheurer, 2015).

However, Gouliouris et al. reported significantly lower removal rates of ampicillin-resistant *E. faecium* (ARE) and VRE than total *E. faecium* (Gouliouris et al., 2019). These removals were, on average, 3.0, 2.7, and 2.5 $\log_{10}$ for all enterococci, ARE, and VRE, respectively (Gouliouris et al., 2019). Ferreira da Silva et al. did not detect VRE among enterococci in a Portuguese WWTP (Ferreira da Silva et al., 2006). *E. faecium* and *E. faecalis* showed resistance to ciprofloxacin, erythromycin and tetracycline, with prevalence values reaching 33 %, 40 % and 57 %, respectively. A positive selection of ciprofloxacin-resistant enterococci was indicated by a significant increase in resistance prevalence ($p < 0.02$) in treated WW compared with raw WW (Ferreira da Silva et al., 2006). Luczkiewicz et al. also found a significantly higher proportion of fluoroquinolone-resistant enterococci in effluent than in influent (Table 5.2.4.-1) (Luczkiewicz et al., 2010).

5.2.4 Examples of emerging AMR-species/subspecies/clones

Bloodstream infections caused by ESBL-E (enterobacteriaceae) have proven to increase the hazard of death, excess length of hospitalisation, and cost significantly compared with susceptible strains (Stewardson et al., 2016). The more humans colonised by ESBL-E the more likely is treatment failure. Woerther et al. reviewed the trend of ESBL-E faecal carriage rates in healthy populations in various parts of the world and showed that community

[3] AMP, VAN, Q/D, TET, RIF, ERY, GEN, CHL, NIT and CIP.

carriage, which was unknown before the turn of the millennium, has increased significantly everywhere, and that CTX-M carriage is evolving globally (Woerther et al., 2013).

ESBLs of class A include mainly TEM, SHV, CTX-M, VEB, and GES enzymes. Among them, the highest number of variants described in recent years corresponds to the CTX-M family. This explosive dissemination of CTX-Ms has been referred to as the "CTX-M pandemic" due to their increasing description worldwide (Cantón et al., 2012).

To date, over 172 CTX-M types have been identified and described (https://www.lahey.org/studies/other.asp), and have been grouped into five clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) (Ramadan et al., 2019).

ESBL-*E. coli* often show multiple co-resistance, complicating first-line treatment of many frequent community infections, such as UTIs. Co-selection with other resistances, especially to fluoroquinolones, aminoglycosides, and sulphonamides, seems to have contributed to the problem in Europe (Coque et al., 2008).

Nosocomial infections have been caused by bacteria with plasmids encoding for ESBL and also carrying resistance to other substances, such as tetracyclines (Sandegren et al., 2012) and fluoroquinolones (Cao et al., 2017; Diwan et al., 2012). Furthermore, it has been shown that resistance plasmids facilitate dissemination within a population and between sectors (Borjesson et al., 2013; Liu et al., 2016; Shen et al., 2016). Of the AMR drivers identified, tetracycline and fluoroquinolones are those that occur in sufficient concentrations in WW to be able to select for bacteria carrying these resistance plasmids (Kraupner et al., 2018; Lundstrom et al., 2016; Sundst et al., 2009). It is, therefore, possible that WWTPs are sites for co-selection of ESBL-E and CPE.

In a Norwegian study (Paulshus et al., 2019b), ESBL-positive isolates of *E. coli* were repeatedly isolated over a 15-month period. The samples were collected on the three first days of each month, with 45 daily samples, and close to 8000 *E. coli* colonies were analysed. From the community sewage (residential area), 10% of 3123 *E. coli* isolates were found to carry ESBL resistance. A few clones dominated the ESBL-positive *E. coli* isolates and whole-genome sequencing of 15 representative isolates from the two phenotypes identified these as two distinct clones belonging to the two globally spread *E. coli* multilocus sequence types (STs) ST131 and ST648 and carrying $bla_{\text{CTX-M-15}}$. Of the ESBL-positive isolates, 37% belonged to ST648, and 7% belonged to ST131. Repeated findings of CTX-M-15-positive ST648 and ST131 over time indicate that these STs are resident in these WW systems and/or circulate abundantly in the community.

Several authors have recommended surveillance of AMR and ARGs in WWTPs to assess the risk of environmental transmission further (Ashbolt et al., 2013; Huijbers et al., 2015; Huijbers et al., 2019; Larsson et al., 2018). Studies reporting ESBL-EC occurrence in WWTP were identified in our literature search, but most were investigating gene occurrence in only influent or only effluent water, or were not geographically relevant for this assessment. Altogether, we identified four studies investigating ESBL-*E. coli* occurrence before and after

treatment. These were from Sweden (Flach et al., 2018; Kwak et al., 2015), The Netherlands (Blaak et al., 2015), and France (Brechet et al., 2014). However, only the study by Blaak et al. directly enumerated ESBL-*E. coli,* whereas the other studies were based on frequencies of the total *E. coli* population determined by screening, with sometimes a proportion of the isolates analysed for ESBL-genes (Blaak et al., 2015). No studies on the removal efficiency of CPE were found.

In the study by Kwak et al., ESBL-E was detected in all samples taken from Henriksdalsverket (Stockholm, serving 640,000 inhabitants) before and after treatment (Kwak et al., 2015). In urban WW an estimated 2.3 % of *E. coli* isolates carried ESBL-genes, whereas in hospital WW the corresponding proportion was 13.6 %. There was no indication of selection for ESBL-producers in the WWTP (Kwak et al., 2015). Similar figures from Besançon, France, were 0.3 % and 7.8 %, respectively, but with large variation between WWTPs (Brechet et al., 2014). The French study indicated a slight increase in the frequency of ESBL-*E. coli,* with 98 % removal of total *E. coli* compared to 94 % of ESBL-*E. coli* (Brechet et al., 2014). Flach et al. noted that the French study was based on relatively few isolates compared to their own in which significant selection during treatment could not be verified, although ESBL-*E. coli* frequencies in Ryaverket (Göteborg, serving 763 000 inhabitants) were 1.8 % in the inflow compared with 2.2 % in the treated WW (Flach et al., 2018).

When directly enumerated, the median ESBL-*E. coli* concentrations in urban WWTPs in the Netherlands were 8.2×10^5 and 1.5×10^3 CFU/L in the influents and effluents, respectively (Blaak et al., 2015). Thus, the mean removal efficiency of ESBL-*E. coli* in Dutch WWTPs was 2.7 $\log_{10}$ (99.8 %), which was slightly higher than total *E. coli* removal. Hospital and nursing home WW in the Netherlands also contained higher numbers of ESBL-EC than urban WW, at 2.0×10^7 CFU/L (Blaak et al., 2015).

Summary of chapter 5.1-5.2.4.

The studies fulfilling the inclusion criteria are summarised in Table 5.2.4-1.

Table 5.2.4-1. Studies fulfilling the inclusion criteria.

Treatment technology	Target bacteria	Target antimicrobials[a]	Removal efficiency	Reference and country
Activated sludge	*E. coli*	AMP, AMC, PIP, PT, CF, CXM, CXMAX, FOX, CPD, CTX, CAZ, FEP, MEM, GM, TM, AMK, SXT, FT, NOR, CIP, OFL, NAL, TET, CHL	2.3 log	(Reinthaler et al., 2003) Austria
Activated sludge	*E. coli*	AMP, CPD, SUL, TMP, CIP, TET, GM	99.7 %	(Flach et al., 2018) Sweden
	E. coli	AMP, SXT, TET, CIP	>2.2 log	(Schwermer et al., 2018) Norway
Activated sludge (tertiary disinfection)	*E. coli*	AMP, CMP, TET	2.8 - >4[b] log	(Turolla et al., 2018) Italy
Not specified	*E. coli*	AMX, TET, CIP	>2 log	(Osinska et al., 2017) Poland
Biological treatment (aeration tank)	*E. coli*	AMK, GM, NN, IPM, MEM, CZ, CXM, CAZ, CTX, FEP, ATM, AMC, TZP, STX, CIP, LVX, MAR	>2 log	(Kotlarska et al., 2015) Poland
Tertiary treatment	Coliforms *Acinetobacter* spp.	*AMK*, AMP, AZT, *CTX*, CHL, CIP, GM, IPM, **NAL**, PIP, STX, TET, TM MAR	1 – 3 log	(Guardabassi et al., 2002) Denmark
Activated sludge	*E. coli*	CTX, CAZ, CPD, TZP, GM, STX, AMK, CMP MAR	2 log	(Korzeniewska et al., 2013) Poland

Activated sludge	*Escherichia* spp.	AMK, GM, **CIP**, STX, TET, **CF**	1.3	(Ferreira da Silva et al., 2007) Portugal
Activated sludge	*E. coli*	AMP, PIP, AMC, ATM, TZP, AMK, TET, CIP, LVX, SXT, IMP, MEM, CTX, CAZ, FEP	99.8 %	(Luczkiewicz et al., 2010) Poland
Conventional (activated sludge)	*E. coli, Klebsiella* spp.	GM, AMP, AMC, SXT, CIP, PIP, TMP, MDR	2 log	(Verburg et al., 2019) Netherlands
Soil filter	*E. coli*	*AMP*, CIP, STX, CTX	2.1 - 3.2	(Scheurer et al., 2015) Germany
Biological treatment (aeration tank)	*Intl1*- and *Int2 positive Enterobacteriaceae*	STR, GM, TOB, AN, NET, KAN, IPM, PIP, **TZP**, AMP, AMC, TIC, **CAZ**, **CF**, **CPD**, **CFZ**, CXM, **CTX**, AZT, SUL, *TMP*, CTR, *NOR*, CIP, *TET*, CMP, NIT, MDR	59 – 99%	(Mokracka et al., 2012) Poland
Secondary, tertiary, UV-disinfection 20 different	*E. faecium*	**VAN**, **AMP**	2.5 - >4 log[d]	(Gouliouris et al., 2019) England
Activated sludge	Faecal enterococci	AMP, **CIP, LVX, MXF**, VAN, TEC, ERM	99.6 %	(Luczkiewicz et al., 2010) Poland
Sand filter	Faecal enterococci	KAN, SXT, TET, GM, NB, NM, CMP, AMP, ERM, CIP, NOR, OFL	>99 %	(Xu et al., 2007) Germany
Soil filter	*Enterococci*	AMP, ERM, VAN, CMP	0.9 - 2.8	(Scheurer et al., 2015) Germany

Tertiary filtration	*Enterococcus* spp.	CIP, VAN	2 log	(Varela et al., 2013) Portugal
Conventional treatment, 14 different	Faecal enterococci	AMP, VAN, Q/D, TET, RIF, ERY, GEN, CHL, NIT, CIP	0.5 – 4 log	(D'Costa et al., 2006) Portugal
Activated sludge	*E. faecium*	**CIP**, ERM, TET, VAN	0.9 – 1.4 log	(Ferreira da Silva et al., 2006) Portugal
Activated sludge	*E. coli*	ESBL, MAR	97%	(Flach et al., 2018) Sweden
Tertiary filtration	*E. coli*	ESBL	99.7%	(Kwak et al., 2015) Sweden
Biological treatment	*E. coli*	**ESBL**	94%	(Brechet et al., 2014) France
Biological treatment	*E. coli*	ESBL MDR	99.8%	(Blaak et al., 2015) Netherlands

[a] Significantly higher proportion of target bacterium to the studied antimicrobial in the outflow is indicated in bold, significantly lower in italics; [b] after disinfection (PAA, UV and NaOCl); [c] *Acinetobacter* spp. [d] after disinfection (UV); Penicillins: Ampicillin AMP, Amoxicillin+Clavulanic acid AMC, Piperacillin PIP, Piperacillin+Tazobactam TZP, Cephalosporins: Cefalothin CF, Cefuroxim CXM, Cefuroxime-Axetil CXMAX, Cefoxitin FOX, Cefpodoxime CPD, Cefotaxime CTX, Ceftazidime CAZ, Cefepime FEP, Carbapenems: Imipenem, IMP, Meropenem MEM, Quinolones: Levofloxacin, LVX, Nalidixic acid NAL, Norfloxacin NOR, Ciprofloxacin CIP, Ofloxacin OFL, Moxifloxacin, MXF; Aminoglycosides: Gentamicin GM, Tobramycin TM, Amikacin AMK, Streptomycin, SM. Neomycin, NM; Glycopeptides: Vancomycin VAN, Teicoplanin TEC. Others: Tetracycline TET, Chloramphenicol CHL, Trimethoprime/sulfamethoxazole SXT, Nitrofurantoin FT, Aztreonam ATM, Erythromycin ERM, Novobiocin, NB

5.2.5 Occurrence of ARB in the effluent water fraction

As indicated under 5.2.4-1 above, there appears to be no significant selection of ARB in WWTPs under European conditions. Although some studies indicated a slight increase in the fraction of resistant bacteria, the absolute reduction over treatment is significant, with removals of between 99 % to 99.9 % of faecal indicator bacteria generally achieved in Scandinavia with secondary treatment, including biological and physico-chemical treatment steps (Table 5.2.5-1). Nevertheless, ARB are still released into receiving waters, which may lead to an increased number of faecal indicators, including resistant populations downstream from WWTP outlets. Tertiary filtration and/or post-disinfection will further reduce indicator bacterial numbers (D'Costa et al., 2006; Hess et al., 2016; Luddeke et al., 2015; Ottoson et al., 2006a; Schwermer et al., 2018). Whereas some studies have indicated a selection potential during ozone treatment, this is not the case for UV (Li et al., 2019). However, process optimisation has yet to be determined, taking into consideration the possibility of photoreactivation and resistant isolates potentially being more likely to reactivate (Guo et al., 2017).

Table 5.2.5-1. Removal efficiencies in WWT processes, expressed as $\log_{10}$ reduction (mean and standard deviation or range) in bacterial numbers between untreated and treated wastewater and sludge respectively.

City/Plant	Treatment	Organism	Removal [mean $\log_{10}$ ± SD]	Reference
Malmö/Klagshamn	Coagulation, activated sludge, rapid filtration	*E. coli* enterococci	2.4 ± 0.4 2.1 ± 0.5	(Ottoson et al., 2006b)
Malmö/Sjölunda	Coagulation, activated sludge	*E. coli* enterococci	2.9 ± 0.4 2.4 ± 0.3	(Ottoson et al., 2006b)
Ryaverket/Gothenburg	Coagulation, activated sludge, extended N-removal	*E. coli* enterococci	2.3 ± 0.9 1.8 ± 0.8	(Flach et al., 2018), (Ottoson et al., 2006b)
Ön/Umeå	Coagulation, activated sludge	*E. coli* enterococci	2.3 ± 0.4 2.2 ± 0.5	(Ottoson et al., 2006b)
Henriksdal/Stockholm	Coagulation, activated sludge, direct filtration including coagulant	*E. coli* enterococci	3.2 ± 0.8 3.2 ± 0.8	(Kwak et al., 2015), (Ottoson et al., 2006a)[a]

Henriksdal/Stockholm	Drum filter, membrane bioreactor	*E. coli* enterococci	5.0 ± 0.9 4.5 ± 1.1	(Ottoson et al., 2006a)[a]
VEAS (Norway)	Sedimentation, biofilm	*E. coli*	2.4	(Schwermer et al., 2018)
BEVAS (Norway)	Sedimentation, activated sludge	*E. coli*	2.4	(Schwermer et al., 2018)
Ryaverket/Gothenburg	MAD 35D 21d storage 11M	*E. coli* Coliforms *E. coli* Coliforms	1.0 – 2.4 2.0 – 3.2 2.6 – 2.9 0	(Rutgersson et al., 2020)[b]
	TAD 55D 9d	*E. coli* Coliforms Enterococci	5.7 5.9 4.5	(Sahlstrom et al., 2004)[c]
	MAD 55D 9d	*E. coli* Coliforms Enterococci	1.6 1.5 2.2	(Sahlstrom et al., 2004)[c]
	MAD 35D 15d	*E. coli* Enterococci	1.9 ± 0.3 0.3 ± 0.5	(Kjerstadius et al., 2013)[d]
Lab-scale study	TAD 55D 7d	*E. coli* Enterococci	> 4.9 > 1.1	(Kjerstadius et al., 2013)[d]
	Pasteurisation 70D 60 min	*E. coli* Enterococci	> 4.9 > 1.4	(Kjerstadius et al., 2013)[d]

[a] Studies in Henriksdal from Ottoson (2006) were made in a pilot plant (1500 PEs); [b] results are based on five samples altogether, two from raw sludge, two digested and one from the stored sludge; [c] based on mean values between raw and digested sludge; [d] studies were made in lab-scale (20 L reactors);

5.2.6 Occurrence of ARB in the treated sludge fraction

Scandinavian data on the inactivation of indicator bacteria in sludge in anaerobic digestion processes are presented in Table 5.2.5-1. As expected, thermophilic digestion is superior to mesophilic in inactivating indicator bacteria. In Norway, sludge must be pasteurised (70 °C

for 60 min) before entering the biogas digester operating at mesophilic temperatures. Inactivation during this time-temperature regimen has proven to be efficient (Kjerstadius et al., 2013).

One explanation for the non-significant selection of ARB during biological treatment in WWTPs is the limited amount of generations that faecal bacteria undergo (Flach et al., 2018). However, sludge digestion processes are considerably longer, with hydraulic retention times of over three weeks in mesophilic digestion processes, providing an opportunity for selection and HGT to occur. Only one study fulfilled the inclusion criteria of our literature search regarding sludge treatment. This study investigated resistance of *E. coli* and other coliform bacteria towards AMP, TET, and CIP after mesophilic digestion (35 °C, 21 days) followed by open-air storage (11 months) (Rutgersson et al., 2020). *E. coli* were reduced by several orders of magnitude during treatment (Table 5.2.5-1) and were not detected at all on plates supplemented with ampicillin or tetracycline. Only a single *E. coli* colony was found on plates with ciprofloxacin. For coliforms (excluding *E. coli*), no ciprofloxacin-resistant colonies were detected in the digested and stored sludge. However, for the other antibiotics, the reduction seen for *E. coli* was not observed, and TET-resistant coliforms were even more abundant in fully treated sludge than in semi-digested sludge. The total number of ARG correlated with human faecal pollution (measured by reads of crAssphage) in sludge samples and decreased during treatment, with no indication of selection (Rutgersson et al., 2020). However, based on five grab samples, these results on sludge treatment should be interpreted with care.

Guardabassi et al. compared the fraction of presumptive coliforms resistant towards AMP, GEN, and TET between raw WW, treated WW, and digested sludge in two large-scale Danish WWTPs over a period of six months. Digested sludge generally contained similar numbers of resistant bacteria to those found in raw WW and in the same resistant:sensitive proportions (Guardabassi et al., 2002). For *Acinetobacter* spp., resistance towards cefotaxime was significantly lower in digested sludge at one of the WWTPs, whereas a larger proportion of tested isolates were resistant towards chloramphenicol than in the raw WW (Guardabassi et al., 2002).

5.3 Occurrence and fate of antibiotic resistance genes (ARG)

5.3.1 General remarks

Concerns have been raised regarding the role of WWTPs as reservoirs and hot spots for ARGs and the possibility of co- and cross-selection by antibiotic residues and other non-antibiotic contaminants, such as metals and biocides (Berglund et al., 2015; Bouki et al., 2013; Karkman et al., 2018; Östman et al., 2017). In the microbe-rich environment of WWTPs, a higher probability of HGT of MGE between faecal bacteria, pathogens, and environmental microbiota would be expected. Furthermore, human strategies to remove

pathogens and organic particles in the treatment process might induce stress responses (SOS response) that promote mutations and HGT (Beaber et al., 2004; Gillings, 2017; Karkman et al., 2018). In recent years, emerging developments within culture-independent methods (e.g., multiplex quantitative PCR, high-throughput sequencing, and metagenomics) have enabled detailed characterization and direct tracing of ARGs in WWTPs, thereby generating a deeper understanding of the complex microbial communities in WW, including their resistomes (Bengtsson-Palme et al., 2016; Karkman et al., 2018). ARGs conferring resistance to most classes of antibiotics have been detected in pre- and post-treatment sewage systems all over the world (Hendriksen et al., 2019; Karkman et al., 2018). The relative abundance in effluent has been observed to be considerably higher than in pristine natural water sources, suggesting that WW represents a significant source of ARG contamination to the receiving water systems (Berglund et al., 2015; Brown et al., 2019; Czekalski et al., 2015; LaPara et al., 2011; Shao et al., 2018). However, there is limited evidence that specific selection occurs in WWTPs (Bengtsson-Palme et al., 2016; Karkman et al., 2018). Interpretation is problematic due to the complexity of WW systems in general, the limited information on expression patterns and origins of ARGs onsite throughout the treatment process, and the lack of harmonized methods and protocols to compare different WWTP systems (Karkman et al., 2018).

5.3.2 Examples of emerging ARGs

Examples of ARGs that have considerable potential for horizontal spread are the *bla* (β-lactam), *mecA* (MRSA), *vanA* (vancomycin), and *qnr* (fluoroquinolone) genes, as well as the plasmid-mediated *mcr-1* gene involved in colistin resistance in *Enterobacteriaceae* (ECDC 2016). The latter is not part of the WHO priority pathogens list (PPL), but is of great clinical concern as colistin is a last-resort antimicrobial agent for infections with MDR gram-negative bacteria.

Other ARGs of clinical relevance that have been frequently monitored in WWTP are the *tet* (tetracycline)*, sul* (sulphonamide), and *erm* (MLSB) genes, all of which may spread horizontally (Auerbach et al., 2007; Berglund et al., 2015; Laht et al., 2014a; Svobodova et al., 2018). The CTX-M genes are often located in MDR regions containing different transposons and insertion sequences. These structures have, in turn, been inserted in narrow and broad host-range plasmids belonging to the same incompatibility groups as those of early antibiotic-resistance plasmids, which frequently carry aminoglycoside, tetracycline, sulphonamide, or fluoroquinolone resistance genes (qnr and/or aac(6')-Ib-cr).

Another example of an emerging ARG proven to disseminate rapidly in clinical settings is New Dehli metallo-ß-lactamase blaNDM-1. This gene was first identified in a New Delhi hospital in 2009, but disseminated worldwide in a very short period of time (Nesme and Simonet, 2015). In *E. coli*, they are frequently carried in well-adapted phylogenetic groups with particular virulence-factor genotypes (Canton and Coque, 2006). This can be exemplified by the uropathogenic *E. coli* ST131 (specifically clone O25b:H4-ST131) with transmission linked to the spread of CTX-M-15 (Schembri et al., 2015).

Furthermore, markers of HGT potential and MDR, such as class 1 integrons (*IntI1*), are frequently included in environmental monitoring of ARGs (Kaushik et al., 2019; Lin et al., 2019; Wen et al., 2016).

Table 5.3.2-1 Examples of ARGs of WHO priority pathogens. Examples of ARGs were retrieved from the literature in January 2020 by searches in PubMed and Google Scholar with the terms "agent name" AND "resistance genes". Note that several of the resistance determinants identified are of intrinsic nature (e.g., mutations in housekeeping genes) that probably spread clonally rather than horizontally through MGE.

Pathogen	**WHO global PPL (2017)[4]**	**Resistance % in EU/EEA 2018[5,6]** **(N)**	**Resistance % in Norway 2018[5,7]** **(N)**	**Examples of ARGs and resistance mechanisms**	**Reference**
Acinetobacter baumanii, carbapenem-resistant	Priority 1: Critical	31.9 %R (N=6501)	0 %R (N=32)	Intrinsic and acquired genes encoding carbapenemases (e.g. *bla* $_{OXA-51}$ *bla*$_{OXA-23}$ and *bla*$_{OXA-24}$)	(Da Silva and Domingues, 2016; Wong et al., 2019)

[4] WHO priority pathogens list (PPL). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO February 2017. (https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/). Accessed December 15 2019.

[5] European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. Stockholm: ECDC; 2019. Accessed 15.12.2019.

[6] Population weighted mean.

[7] NORM/NORM-VET 2018. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø / Oslo 2019. ISSN: 1890-9965 (electronic).

Pathogen	WHO global PPL (2017)[4]	Resistance % in EU/EEA 2018[5,6] (N)	Resistance % in Norway 2018[5,7] (N)	Examples of ARGs and resistance mechanisms	Reference
Enterobacteriaceae carbapenem resistant, ESB-producing	Priority 1: Critical	Carbapenems: *E. coli:* 0.1 %R (N=149725) *K. pneumoniae:* 7.5 %R (N=37824) Aminopenicillins: *E. coli:* 57.4 %R (N=131969) Third-generation cephalosporins: *E. coli:* 15.1 %R (N=150989) *K. pneumoniae:* 31.7 %R (N=38122)	Carbapenems: *E. coli:* <0.1 %R (N=3879) *K. pneumoniae:* 0.1 %R (N=736) Aminopenicillins: *E. coli*: 42,3 %R (N=3880) Third-generation cephalosporins: *E. coli*: 6.8 %R (N=3879) *K. pneumoniae*: 7.5 %R (N=737)	Acquired, plasmid-borne genes encoding OXA-carbapenemase (*e.g.* $bla_{OXA\text{-}48}$) and New Dehli metallo-β-lactamase-1 ($bla\text{-}_{NDM\text{-}1}$) conferring resistance to penicillins, cephalosporins, and carbapenems. Various $bla_{CTX\text{-}M}$ genes located on plasmids adapted to *Enterobacteriaceae* encoding extended-spectrum β – lactamases.	(Nordmann et al., 2011; Poirel et al., 2012; Woerther et al., 2013)
Pseudomonas aeruginosa carbapenem-resistant	Priority 1: Critical	12.8 %R[8] (N=19119)	2.4 %R[8] (N=250)	Integrons carrying determinants for carbapenem resistance such as bla_{VIM} *and* bla_{OXA} genes	(Kaushik et al., 2019; Rojo-Bezares et al., 2014)

[8] Combined resistance to three or more antimicrobial groups among piperacillin+/-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems

Pathogen	WHO global PPL (2017)[4]	Resistance % in EU/EEA 2018[5,6] (N)	Resistance % in Norway 2018[5,7] (N)	Examples of ARGs and resistance mechanisms	Reference
***Campylobacter* spp.** fluoroquinolone-resistant	Priority 2: High	Not described	Not described	Point mutation in the QRDR region within the *gyrA* gene. Substitutions in the 23S rRNA gene. *ermB* encoding a ribosomal methylase in the 23S rRNA gene leading to decreased binding of macrolides.	(Sierra-Arguello et al., 2018; Whelan et al., 2019) (Bolinger and Kathariou, 2017)
E. faecium vancomycin-resistant	Priority 2: High	17.3 %R (N= 15739)	2.3 %R (N=171) Veterinary isolates: in 2018, no VRE were detected in poultry production (decline from previous years)	Acquired operons that alter the nature of peptidoglycan precursors (e.g., the *vanA* operon). The operon is carried by the Tn3 family transposon Tn1546, located on the chromosome or on plasmids.	(Garcia-Solache and Rice, 2019; Sanderson et al., 2016)
Salmonellae fluoroquinolone-resistant	Priority 2: High	Not described	Not described in human isolates. Note: Norway is considered virtually free of *Salmonella* spp. in livestock populations.	Mutations in quinolone-resistance-determining regions (QRDR) of chromosomal *gyr* and *par* genes. Acquired plasmid-borne resistance genes such as *qnr* genes (*qnrA, qnrB, qnrS, qnrC, qnrD), aac(6')-Ib-cr, oqxAB* and *qepA.*	(Cuypers et al., 2018)

Pathogen	WHO global PPL (2017)[4]	Resistance % in EU/EEA 2018[5,6] (N)	Resistance % in Norway 2018[5,7] (N)	Examples of ARGs and resistance mechanisms	Reference
S. aureus methicillin-resistant (MRSA), vancomycin-intermediate and resistant (VRSA)	Priority 2: High	MRSA: 16.4 %R (N=72059) VRSA: Not described	MRSA: 0.9 %R (N=1547). Note: Findings of MRSA in animal population are rare. No findings in swine population in 2018 VRSA: Not described	MRSA: Exogenous *mec* genes encoding PBPs with low binding affinity to β-lactams (e.g., *mecA*). *mecA* is carried in a mobile genetic element (SCC). VRSA: The *vanA* operon encoded on transposon Tn1546 acquired from enterococcal plasmids	(McGuinness et al., 2017; Miragaia, 2018; Sanderson et al., 2016)
Helicobacter pylori clarithromycin-resistant	Priority 2: High	Not described	Not described	Point mutations in 23S rDNA	(Redondo et al., 2018)
Neisseria gonorrhoeae cephalosporin-resistant, fluoroquinolone (FQ)-resistant	Priority 2: High	Not described	FQ resistance (ciprofloxacin): 68.9 %R (N=315) Cefixim resistance: 1.3% (N=315)	FQ: Mutations in *gyrA* Cephalosporins: *penA* XXXIV encoding PBP2s with reduced affinity to cephalosporins	(Grad et al., 2014; Hall et al., 2019)
***Shigella* spp.** fluoroquinolone (FQ)-resistant	Priority 3: Medium	Not described	Not described	Accumulated mutations in chromosomal *gyrA* and *parC* genes (clonal). Plasmid-mediated R genes are uncommon.	(Chung The et al., 2019)
Streptococcus pneumoniae **penicillin-non-susceptible**	Priority 3: Medium	Penicillin resistance: Weighted mean not given.	Penicillin resistance: 5.0 %NWT (N=500)	Mosaic *pbp* genes that encode modified penicillin binding proteins (PBPs).	(Cornick and Bentley, 2012)

Pathogen	WHO global PPL (2017)[4]	Resistance % in EU/EEA 2018[5,6] (N)	Resistance % in Norway 2018[5,7] (N)	Examples of ARGs and resistance mechanisms	Reference
Haemophilus influenza ampicillin-resistant	Priority 3: Medium	Not described	14.3% of isolates were resistant (very low number of isolates in total; n=14 makes interpretation difficult)	Genes encoding enzymes that hydrolyse bet-lactams, e.g. *blaTEM-1* (acquired). There has been a rise in cases due to intrinsic mechanisms (e.g., mutations in PBPs).	(Heinz, 2018)

5.3.3 Occurrence of ARGs in the effluent fraction

ARGs of most antibiotic classes have been detected in WWTP systems (Karkman et al., 2018; Pazda et al., 2019). A recent metagenomic study found that ARGs encoding resistance to macrolides, tetracyclines, aminoglycosides, beta-lactams, and sulphonamides were most abundant in raw untreated sewage sampled from numerous cities across the world (Hendriksen et al., 2019). Table 5.3.3-1 summarizes studies describing the occurrence of various ARGs in European WWTPs. The abundance, diversity, and net change between influent and effluent vary between studies and sites making it difficult to generalize results (Barancheshme and Munir, 2017; Karkman et al., 2019; Krzeminski et al., 2020; Pei et al., 2019). Furthermore, there is apparently no harmonized method for monitoring ARGs in WW and this hampers inter-study comparison.

There are limited data on the occurrence of ARGs in Norwegian WW. Schwermer et al. analysed samples from two municipal WWTP in Oslo and detected several markers of resistance towards commonly used antimicrobials in Norway (Schwermer et al., 2018). However, there was no clear correlation between antimicrobial usage data and the corresponding ARGs. Interestingly, ARGs determining resistance towards last-resort antimicrobials (e.g., colistin) were found despite these being rarely used in Norwegian hospitals.

Changes in ARGs between influent and effluent might be given as absolute concentration (per volume) or relative to bacterial load indicated by the 16S rRNA gene (relative abundance). Most studies have reported that absolute concentrations of ARGs decrease during sewage treatment, probably as a result of the dilution effect and reduced general bacterial loads throughout the treatment process. The tendency of relative abundances is, however, ambiguous (Lee et al., 2017; Rafraf et al., 2016). Some studies have found an increase in relative abundance of ARGs in effluent WW, and have speculated that there might be a selective advantage for ARGs (and ARBs) in some WWTP and under certain operating conditions (reviewed by Karkman et al 2018). However, the majority of twenty-five independent studies (reviewed by Li et al.) showed a net relative reduction in ARGs suggesting that, overall, WWT tends to reduce the amount of ARGs in effluent (Li et al., 2019). This is supported by Pallares-Vega et al. who investigated 62 Dutch WWTPs and applied a thorough statistical analysis (Pallares-Vega et al., 2019). They found that concentrations of ARG were removed to a similar extent, or even more, than the total bacteria (measured as 16S rRNA). However, a significant increase in the relative abundance broad-host-range plasmids (IncP-1) was noted after treatment. Recent results suggest that reduction of ARGs is related to the elimination of faecal material (Karkman et al., 2019). The effect of various treatment methods on removal of AMR (ARBs and ARGs) has recently been reviewed (Hiller et al., 2019; Krzeminski and Popowska, 2020). While ultrafiltration processes seem to be suitable for removal of ARG, as shown by Krzeminski et al. (2020), the efficacy of various disinfection methods is less clear (Krzeminski et al., 2019). Liu et al showed that chlorine disinfection of WW increased intracellular and extracellular ARG (Liu et al., 2018).

Few studies have investigated the effect of treatment methods on cell-free naked DNA, although conventional methods are expected to have a limited effect. Zhang et al. investigated the effect of WWT (a combination of biological treatment, sludge settling, membrane filtration, and disinfection), on ARGs in the cell-free and cell-associated fractions (Zhang et al., 2018). They found that ARGs in the cell-free fraction increased during the treatment process compared with the cell-associated fraction. Furthermore, the study indicated that cell-free ARGs persisted in the effluents for weeks. Krzeminski et al. suggested that membrane filtration (1 kDa cut off) combined with UV irradiation might be an effective measure to prevent release of plasmid-bound ARGs to the receiving water environment (Krzeminski et al., 2020). Overall, current knowledge indicates that membrane filtration has the greatest potential in removing ARGs from WW.

Although several recent studies have investigated the occurrence of ARGs in effluents, there are still considerable knowledge gaps on the effects of different WWT methods on ARG abundance and comprehensive studies are therefore warrantied.

Table 5.3.3-1. Examples of ARGs measured in effluents from European WWTPs.

Country	WW treatment	Target ARGs	Detection method	Detected in effluent (net change influent-effluent)	Reference
Germany	Mechanical separation/sedimentation + ozonisation*	vanA (vancomycine) blaVIM (imipenem), ermB (erythromycin)	qPCR	Increase of *vanA* and *bla*VIM. Decrease of *ermB*	(Alexander et al., 2016)
Germany	Conventional treatment + ultrafiltration or ozonisation	sul1, blaTEM, tetM, CTX-M, CTX-M-32, blaOXA-48, blaVIM, CMY2, vanA, mcr-1, blaNDM.	qPCR	All targets except vanA, mcr-1 and blaNDM, bla VIM, were detected in effluent. UF reduced ARGs by an average of 5 log units.	(Hembach et al., 2019)
Sweden	Not specified	Genes targeting all major AB classes	Metagenomics	The majority of genes were decreased. Increase in QAC and trimethoprim	(Bengtsson-Palme, 2016)

Sweden	Not specified	*sulI* (sulphonamide), *dfr1* (trimethoprim), *ermBn* (macrolide/lincosamide/streptogramin B), *tetA* and *tetB* (tetracycline), *vanB* (vancomycin), qnrS (quinolone), *intI1*, the integrase gene on class 1 integrons.	qPCR	All targets except *qnrS* were detected in effluent. Effect of treatment was not assessed.	(Berglund et al., 2015)
Sweden	Sand trap, settling, activated sludge	*mecA* (methicillin)	qPCR	Decrease of *mecA*	(Borjesson et al., 2009)
Spain	Activated sludge	*mcr-1* (colistin)	qPCR	Decrease	(Lekunberri et al., 2017)
Norway	Not specified	bla_{TEM-1D}, $bla_{CTX-M-15}$, $bla_{CTX-M-32}$, bla_{KPC-3}, bla_{OXA-48}, bla_{OXA-58}, *Int1, mcr-1, sul1, tetM,*	qPCR	All targets detected. Highest concentrations of *Int1* and *sul1*.	(Schwermer & Uhl, 2018; Schwermer & Uhl, 2019))
Norway	Mechanical treatment	bla_{TEM}, *mecA, qnrS, ermB, aph(3`)-IIa, aph(3`)-IIIa, aac(6`)/aph(2``) sulI, tetA* and *dfrA1*	PCR	The following targets were detected: *blaTEM, aph(3`)-IIIa, aac(6`)/aph(2``), qnrS* and *ermB*)	(Nordgård et al., 2016)
Cyprus, **Portugal,** **Germany**	Activated sludge Membrane bioreactor	*vanA*, bla_{TEM}, *qnrS, sul1*, $bla_{CTX-M-32}$ and *intI1*	qPCR	*Int1* and *sul1* were most abundant, detected at all locations	
Czech republic	Sedimentation, chemical treatment/coagulation, biological treatment	*tet, bla, erm, sulf, int1*	qPCR	Decrease of *tet, bla, erm, sulf*. Increase of *int1*.	(Svobodova et al., 2018)

Finland	Primary mechanical treatment and secondary biological treatment (activated sludge) followed by tertiary purification treatment.	*sul1, sul2, tetM, tetC, bla$_{OXA-58}$, bla$_{shv-34}$, bla$_{CTX-M-32}$,*	qPCR	Net decrease or no significant change of target abundances in effluent, except for *bla$_{shv-34}$*	(Laht et al., 2014b)
Holland	Conventional treatment	*ermB, tetM, sul1, sul2, qnrS, bla$_{CTX-kM}$, intl1, korB*	qPCR	Decrease (1.76-2.65 logs) but still release on average 10^6 copies/L	(Pallares-Vega et al., 2019)

*only effect of ozonisation was evaluated.

5.3.4 Occurrence of ARG in the treated sludge fraction

Sewage sludge may act as a reservoir of ARGs that can disseminate in soil when used as fertilising product in agriculture. The nutrient-rich environment of sludge, combined with high microbial diversity and the presence of residues of antimicrobials, metals, and biocides, may promote development of co- and cross-resistance (Östman et al., 2017). A series of studies have investigated and reviewed the presence of ARGs in treated sludge to try to elucidate the resistance dynamics of WWTPs (Auerbach et al., 2007; Bengtsson-Palme, 2016; Galler et al., 2018; Guo et al., 2017; Karkman et al., 2018; Lin et al., 2019). Aerobic activated and anaerobically digested sludge from a municipal WWTP in China was sampled and investigated for the abundance of ARGs and MGEs (plasmids, transposons, insertion sequences, integrases) using metagenomic sequencing (Guo et al., 2017). Although there were differences in the resistome between the two sample types, ARGs and MGEs were highly abundant in both.

In a study of two WWTPs in China, several ARGs conferring resistance to tetracyclines, sulphonamides, quinolones, or macrolides were found to be enriched (relative to 16s rRNA genes) through the treatment process, with substantial release through dewatered sludge (Mao et al., 2015).

According to Lin et al., the abundance of ARGs (*sul* and *tet* genes) and *Intl1* was higher in treated sewage sludge than in animal manure, food-derived compost, and soil (Lin et al., 2019).

Sludge samples from Swedish municipal WWTPs were examined for the abundance of ARGs at different stages of the treatment process using shotgun metagenomics (Bengtsson-Palme, 2016). The abundance of several integrases was higher in treated sludge than primary sludge indicating an increased potential of HGE. Furthermore, ARGs against beta-lactams, macrolides, and tetracyclines were enriched in the treated sludge, some of which are emerging and previously uncommon in isolates from Swedish patients (e.g., *bla$_{OXA-48}$*).

Thus, there are indications that conventional sludge treatment methods are not particularly efficient at ARG removal. We are not aware of any ARG data from studies in sludge in Norway, but it is likely that the situation is very similar to the one in Sweden (Larsson et al., 2018; Rutgersson et al., 2020).

Generally, from the few publications available, the concentration of ARG in treated sewage sludge seems to be higher than in the untreated sewage. This is most probably due to the adsorption of DNA on particulate matter, which got concentrated in the sludge. It is in line with the findings of lower concentrations in the treated sewage fraction (as reviewed by Li et al., 2019), compared to the untreated sewage.

No data are available at all regarding any effect of different sludge treatment methods on the concentration of ARG in the treated sludge. And there is currently no evidence at all whether elevated temperatures under e. g. thermal hydrolysis will be sufficient for ARG inactivation and to bring about lower ARG concentrations.

5.4 ARB and ARG in hospital wastewater

Investigations related to emergence, occurrence, and dissemination of ARB and ARG in hospital WW are very diverse in methods and the way that the data are presented. The short review below gives an overview of the diversity of the studies.

The results of a study by Voigt et al. highlight the role of hospital WW for the dissemination and development of MDR as *P. aeruginosa*, resistant against 3rd-generation cephalosporins, was mainly detected in clinical effluents (Voigt et al., 2020). King et al. showed that hospital effluents in South Africa contain antibiotic-resistant *Klebsiella* spp. and may pose a risk to neighbouring informal communities if inadequately treated (King et al., 2020). According to a study by Verburg et al., bacterial AMR profiles in WW mirrored antimicrobial consumption in the relevant locations, and were highest in the hospital setting (Verburg et al., 2019). However, the contribution of hospital WW to AMR found in the WWTP was below 10 % for all antimicrobials tested. The study of Tesfaye et al. demonstrated that *Enterobacteriaceae* in WW from hospitals are resistant to commonly used antimicrobials (Tesfaye et al., 2019). Hospital effluents contained more MDR bacteria, posing a significant potential public health threat through dissemination to downstream waterbodies. Paulshus et al. monitored bacterial diversity in different WW in the Oslo area, and found the highest concentration of antibiotic resistance in hospital WW (Paulshus et al., 2019a). However, surprisingly high concentrations were also found in WW from a residential area. The relative contribution of hospital effluents seemed low in terms of dissemination of ARB to the WWTP. The results of a study by Khan et al. indicate that hospital WW were reservoirs of most ARG and contribute to the diversity of ARG in associated natural environments (Khan et al., 2019). However, this study also suggests that other factors may have minor contributions to the prevalence and diversity of ARG in natural environments.

In a study by Cahill et al. hospital effluent in an urban area in Ireland was examined. It was concluded that releasing untreated hospital effluents may fuel dissemination of AMR in the environment (Cahill et al., 2019). Further studies showed that significant quantities of ARB are carried to municipal WWTP by untreated hospital effluents (Haller et al., 2018; Lamba et al., 2017; Nasri et al., 2017). According to Buelow et al. hospital sewage was richest in human-associated bacteria and contained the highest relative levels of ARG compared to WW sampled at other sites (Buelow et al., 2018). Nevertheless, the relative abundance of ARG was comparable in the influent of WWTPs, both with and without hospital sewage, suggesting that hospitals do not contribute significantly to the quantity and diversity of ARG in the sewerage system under investigation.

Wang et al. determined the concentrations of antibiotics, ARB, ARG, and MGE in untreated hospital WW in China (Wang et al., 2018). On the basis of their results, the authors suggest that highly abundant antibiotic-resistant pathogens and highly mobile ARG already exist in the human body, and that their release from hospitals without effective treatment poses high risks to environments and human health.

In bacterial isolates from hospital wastewater in Ethiopia, Dires et al. found 100 % of *Salmonella* isolates were resistant to ampicillin and 75 % to doxycycline, erythromycin, ceftazidime, cefoxitin, and chloramphenicol (Dires et al., 2018). Among *E. coli* isolates, 82 % were resistant to ampicillin and 73 % to cotrimoxazole and amoxicillin-clavulanic acid. In a study by Szekeres et al., the occurrence and abundance of several antibiotics and ARG were investigated, as well as bacterial community composition in effluents from different hospitals located in Cluj County, Romania (Szekeres et al., 2017). High concentrations of β-lactam antibiotics, glycol-peptides, and trimethoprim were detected.

Lucas et al., 2016 investigated veterinary hospital effluents and concluded that it was not possible to establish a clear link between concentrations of antibiotics and corresponding ARG in WW (Lucas et al., 2016). Thus, there seemed to be other factors that should be taken into consideration besides antibiotic concentrations that reach aquatic ecosystems in order to explain the emergence and spread of AMR.

A study by Rodriguez-Mozaz et al. revealed a higher occurrence of some antibiotics, such as ciprofloxacin and ofloxacin, in hospital WW samples than in the influents of a WWTP (Rodriguez-Mozaz et al., 2015). However, no significant differences were found between both locations in relation to ARG. The WWTP was unable to remove antibiotics and ARG from urban effluents totally, and therefore a proportion were released into the environment.

WW discharged from clinical isolation and general wards at two hospitals in Singapore was examined by (Le et al., 2016). The results showed the widespread occurrence of ARB, ARG, and genetic elements, potentially favouring the transfer of AMR gene cassette arrays in hospital WW.

Herrmann et al. considered the emission of active pharmaceutical ingredients from health institutions in Germany using a consumption-based approach (Herrmann et al., 2015). They

found that the contribution of health institutions to total WW discharge of those pharmaceuticals was very low compared with households. Kümmerer also showed that hospitals in Germany are not the main source of resistant bacteria in municipal sewage, as they were also present in municipal sewage not receiving hospital effluent (Kümmerer 2009). Due to the extensive out-of-hospital use of antibiotics, it is probable that general society is responsible for the main input of ARB into the environment. This can be attributed to resistance developing mainly during medication. In contrast, on basis of their data, Pauwels and Verstraete showed that in hospital effluents the number of pathogens with acquired resistance is 2 to 10 times higher than in private household sewage (Pauwels and Verstraete, 2006). Similarly, Schwartz et al. detected resistant species only in hospital WW and not in municipal sewage (Schwartz et al., 2003). Furthermore, according to results obtained by Bendt et al., hospital effluents are the main source of ARB input into sewage systems, although the effluent volume share is only 1 % of the total volume of sewage water (Bendt et al., 2002).

A concentration ranging between < 1 % and 40 % of ARB in Indian hospital effluents was determined by Chitnis et al., whereas the concentration in urban sewage from a residential area was considerably below 1 % (Chitnis et al., 2000). Guillaume et al. determined a share of 12% of tetracycline-resistant bacteria in the activated sludge in a facility treating hospital effluent (Guillaume et al., 2000). A similar study was performed by van Overbeek et al., who found almost 5% streptomycin-resistant pathogens in the activated sludge of a hospital WWTP (van Overbeek et al., 2002). Rizzo et al. analysed imipenem resistance, measured as blaVIM genes, in WW samples from residential areas, a hospital, and WWTPs, and reported that the highest abundances occurred in hospital WW (Rizzo et al., 2013). Sharma et al. investigated the bacterial load of healthcare liquid waste generated in central hospitals in Nepal). (Sharma et al., 2016). They found alarmingly high numbers of MDR bacteria. Galvin et al. demonstrated that hospital WW had a higher abundance of cephalosporin and quinolone resistance than municipal wastewater without hospital WW contributions (Galvin et al., 2010).

In addition, some studies have considered the transfer and activation of resistance in the presence of antibiotics in different WW, such as hospital effluents. For example, a significant transfer of resistance to antibiotics like gentamycin, tetracycline, ciprofloxacin and erythromycin could not be proven for antibiotics concentrations up to 100 µg/L (Ohlsen et al., 2003). A transfer of resistance genes between gram-positive bacteria within the aquatic environment is regarded as likely.

5.5 Antifungal resistance in wastewater, effluent water, sludge

No data regarding antifungal resistance in WW, effluent water, and sludge in Norway were available at the time of this review.

5.6 Biocides (disinfectant) resistance in wastewater, effluent water, sludge

No data regarding biocides (disinfectants) resistance in WW, effluent water, and sludge in Norway were available at the time of this review.

5.7 PTM resistance in wastewater, effluent and sludge

The MICs for 15 *E. coli* isolates regarding tolerance towards Cu and Zn were determined using agar plates supplemented with $CuSO_4$ and $ZnSO_4$. The MICs for control strains were 16 mM for Cu^{2+} and 4 mM for Zn^{2+}, while MICs for the 15 isolates were similar for all and measured 16 mM for Cu^{2+} and 8 mM for Zn^{2+}. The metal tolerance did not increase in the isolates collected later in the study. These results indicate that local isolates were equally tolerant to Cu as the two globally spread *E. coli* multi-locus STs, ST648 and ST131, and slightly less tolerant to Zn. The levels of Cu and Zn in the WW are probably not high enough to confer any selective pressure that explains the repeated findings of these STs in the wastewater (Paulshus et al., 2019a).

With the exception of the data presented above, no information regarding PTM resistance in WW, effluent, and sludge in Norway were available at the time of this review.

5.8 Summary of Chapter 5

5.8.1 Summary of ARB and ARG in wastewater

- ARB are reduced in WWT to the same extent as sensitive strains of the same species, with no evidence of selection during treatment.
- In two studies, higher frequencies of *E. coli* resistant to cephalosporins were observed in the effluent water than in the influent.
- VRE occurrence was significantly higher in WWTPs receiving hospital WW.
- ESBL-E occurrence was higher in hospital WW than urban WW, but did not affect concentrations in municipal WWTPs to the same extent as VRE.
- No CPE-specific studies fulfilled the criteria for inclusion in this review. For these bacteria, hospital WW might affect concentrations in municipal WWTPs.
- Sludge treatment technologies efficiently reduce enteric bacteria in the solid fraction. However, due to a limited number of studies performed under relevant conditions, it is not possible to draw any conclusions on the selection potential in these processes.

Although several authors stress that WWTPs are reservoirs of VRE transmission to the environment, especially when receiving water from hospitals (Garcia et al., 2007; Gouliouris et al., 2019), the environmental pathway is likely minor compared with transmission within healthcare settings. For resistant Gram-negative bacteria, hospital effluent does not have the same influence on urban WW, considering high carriage rates and the major use by

outpatients (Blaak et al., 2014; Korzeniewska and Harnisz, 2013; Schwermer et al., 2018). This is especially true of antibiotics that can co-select for ESBL and carbapenemase genes (Coque et al., 2008).

There appears to be no significant selection of ARB in WWTPs under European conditions. Although some studies indicated a slight increase in the fraction of resistant bacteria, the absolute reduction over treatment is significant, with removals of 99 % to 99.9 % of faecal indicator bacteria generally achieved in Scandinavia with secondary treatment, including biological and physico-chemical treatment steps. Nevertheless, ARB are still released into receiving waters, which may lead to an increased number of faecal indicators, including resistant populations, downstream from WWTP outlets.

Whereas biological treatment has been the most efficient step in removing indicator bacteria in conventional treatment in many studies (table 5.2.4-1), activated sludge (or similar technologies) has also been reported as a main place for horizontal transfer of ARGs (Ashbolt et al., 2013; Zhang et al., 2009). In the activated sludge, where the bacterial density is very high, the genes responsible for AMR may spread through bacterial populations via plasmids and a variety of MGE, causing an increase in antibiotic resistance of microorganisms (Mao et al., 2015; Zhang et al., 2009). However, based on the studies included, there is no evidence of such an increase during the biological treatment step.

Clearly, 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 draw any conclusion regarding potential selection of phenotypic resistance during sludge treatment. In general, thermophilic treatments or treatments at high pH result in a higher reduction than mesophilic treatment and/or storage. Studies by Tian et al., 2016 and 2019 indicated more efficient relative removal of ARGs in thermophilic processes due to reduced HGT (i.e., fewer MGE and receiving bacterial species) (Tian et al., 2019, Tian et al., 2016). In the review by Li et al., ARGs were reduced relative to total 16S rRNA gene counts in 21 of 27 observations in anaerobic digestion but increased in 6 (Li et al., 2019).

A broad range of ARGs, representing most antimicrobial categories, have been identified in WWTPs worldwide, with marked inter-study variations in abundance, diversity, and net change. The abundance of ARGs has been observed to be considerably higher in effluent than in pristine natural water sources, suggesting that WW represents a significant source of ARG contamination to the receiving water systems. Of greatest concern are those ARGs that are able to disseminate rapidly by horizontal transfer and are known to cause treatment failure in human infections. Only a few studies have been conducted to characterize the situation in Norway. From these studies it appears that clinically relevant ARGs can be found in Norwegian WW, even emerging ARGs against last-resort antibiotics such as colistin. The source of colistin-resistant bacteria was unknown and not identified. It is also evident that conventional treatment processes are not particularly efficient in removal of naked ARG. Most European studies report a net reduction in ARG abundance in WW effluents compared

to influents. However, when adjusting for total bacterial load (16S rRNA) the literature is rather inconclusive. Thus, based on current literature, it is impossible to draw any conclusions on potential selection of ARGs in WWT processes, as well as any interactions with metals and biocides under Norwegian conditions.

At the time of this opinion no data were available regarding antifungal resistance, and biocide (disinfectant) resistance in WW, effluent water, and sludge in Norway, and only limited data were available regarding PTM resistance.

6 Antimicrobial agents in effluent water, sludge and soil

6.1 General remarks

This chapter presents data on the occurrence of antimicrobial agents released into the environment from the WWTPS; namely effluent wastewater and sludge. Furthermore, the Norwegian regulations of use of sludge regarding content of antimicrobial agents are reviewed. Finally, some data on degrading of different antimicrobials in soil are given.

6.2 Occurrence of antimicrobial agents in effluent wastewater and sludge

6.2.1 Antibiotics

There are no available data on antimicrobials in effluent sewage from Norwegian WWTPs, and sludge has only recently been analysed for some antimicrobials (Blytt and Stang, 2019). In that study, 80 samples from 18 different WWTPs were analysed for 13 antimicrobials, and the presence of Azithromycin (mean conc.: 32.1 µg/kg), Clindamycin (7.8 µg/kg), Clindamycin sulphoxide (3.78 µg/kg), Clarithromycin (12.1 µg/kg), 1-Acetylsulphamethoxazole (0.73 µg/kg), Sulphadiazine (4.42 µg/kg), Sulphamerazine (1.41 µg/kg), Sulphamethizole (1.23 µg/kg), Sulphamethoxazole (2.2 µg/kg), Sulphapyridine (37.5 µg/kg), and Trimethoprim (8.8 ug/kg) was recorded. However, two sulpha-compounds (N4-acetyl-sulphamethoxazole and Sulphamethazin) were below the limit of quantification.

6.2.2 Antifungal agents

There are no available data on antifungal agents in sewage effluent or sewage sludge from Norwegian WWTPs.

6.2.3 Biocides (disinfectant agents)

There are no available data on biocides in sewage effluent from Norwegian WWTPs, but sewage sludge has been monitored for biocides, such as phenols, organotin compounds, and triclosan (Blytt and Stang, 2019). Phenols constitute a diverse group, and some, like Bisphenol A and nonylphenol (and their etoxilates) have been measured in 2006, 2012, and 2018, showing more than a doubling for Bisphenol A (mean conc. 2018: 1605 µg/kg) and a >90% reduction in nonylphenol+etoxilates since 2016 (mean conc. 2018: 4127 µg/kg). Organotin compounds have been measured in sewage sludge in 2012 and 2018 at four WWTPs. Concentrations have roughly doubled during this period and the mean

concentration at the last measurement was 75 µg/kg, mainly di-butyl tin. Triclosan concentrations in sludge vary greatly among WWTP, peaking at >4000 µg/kg in two plants, while the median value for 18 WWTPs was 400 µg/kg. Average concentrations of triclosan in sewage sludge sampled in Norway have decreased by 50 % since 2006, and by 33 % since 2012.

6.2.4 Potentially toxic metals (PTM)

Norwegian WWTPs monitor a range chemicals and elements in in-coming WW, effluents, and sludges, mainly as a means of verifying the performance of the treatment methods used, but also to survey the quality of incoming water and the sludge that is used for soil amendment. The most closely monitored substances are PTM (heavy metals). Seven metals (see below) are routinely analysed in sewage sludge end-product, and annual inventories are available for most WWTPs. Summarized data for the last 25 years shows declining trends for some metals (Cd, Cu, Hg, Pb), while concentrations of other metals (Cr, Ni, Zn) have remained stable during this period (Fig 6.1.4-1; SSB 2018; (Statistisk sentralbyrå, 2018).

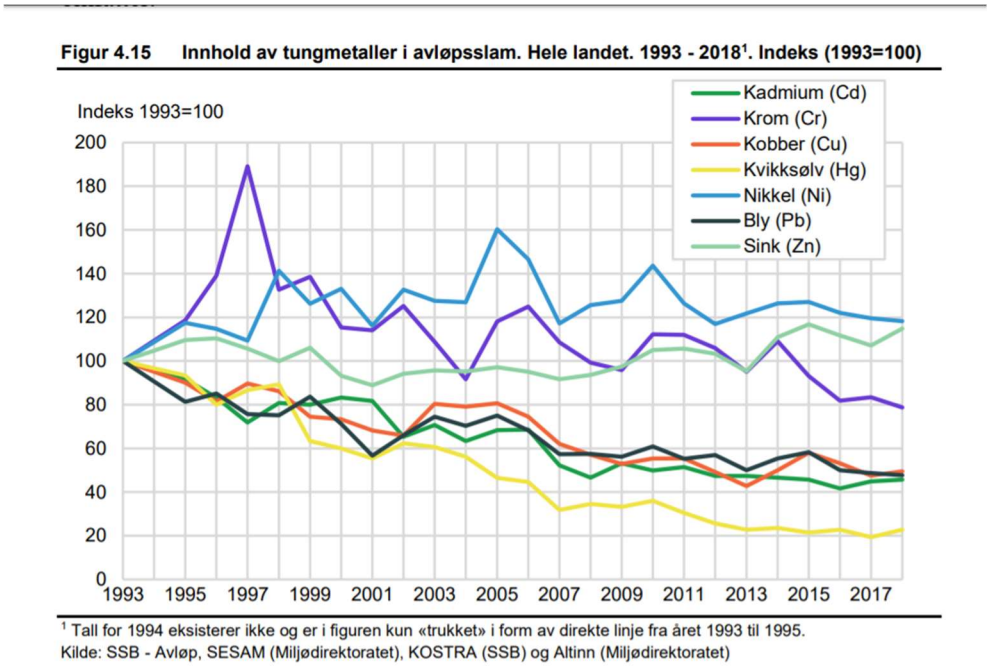

Figure 6.2.4-1. Relative mean concentrations of heavy metals in Norwegian sewage sludge during the last 25 years relative to concentrations measured in 1994 (1994= index 100). Sources: Statistics Norway and Norwegian EPA.

Table 6.2.4-1. Mean concentrations of heavy metals in Norwegian sewage sludge in 2018 (source: Norwegian EPA/Miljødirektoratet).

Elements	Mean concentration* (mg/kg DW)	Lower 95% confidence interval limit	Upper 95 % confidence interval limit	Upper limit for use in agriculture (Class II) (mg/kg DW)
Cd	0.6	0.5	0.6	2
Cr	16.9	14.4	19.7	100
Cu	165	146	184	650
Hg	0.3	0.3	0.4	3
Ni	12.9	11.4	14.5	50
Pb	13.8	11.7	15.9	80
Zn	391	347	436	800

*Mean concentration corrected for mass contribution

WWTPs remove a large proportion of most heavy metals. As an example, the largest Norwegian WWTP, VEAS, reported removal rates from 12-92 %, varying strongly with the metal in questions (Table 6.2.4-2; VEAS 2019) (VEAS, 2019). In comparison, rivers and other run-off represent inputs of metals to coastal waters amounting to 128 kg Hg, 2 tonnes of Cd, 23 tonnes of As, 39 tonnes of Pb, 35 tonnes of Cr, 238 tonnes of Ni, 5767 tonnes of Zn, and 1251 tonnes of Cu for the year 2016 (Skarbøvik et al., 2017). Comparison of these figures with those in the table below, demonstrates clearly that treated WW represents a very minor fraction of the anthropogenic load on coastal waters (2.6 % for Hg, 0.2% for Cd, 0.002% for As, 0.1 % for Pb, Cr, and Ni, and 0.03 % for Zn and Cu).

Table 6.2.4-2. Removal rates of PTM in WWTPs.

Elements	Incoming (kg/yr)	Discharged (kg/yr)	Removal (%)
As	86	46	47
Pb	238	39	84
Cd*	8.4	4.5	46
Cu	3584	434	88
Cr*	160	48	70
Hg*	3.9	0.33	92
Ni	281	247	12
Zn	6755	1857	73

* 90 % of the samples <Limit of Quantification (cons -> 50 % of Limit of Quantification)

6.3 Regulation of use of sewage sludge regarding content of antimicrobial agents

The use of sewage sludge in soil and fertilising products is strictly regulated in Norway (for more information see Section 5). Maximum allowable concentrations of contaminants are below those of the EU and most of its member states. The regulation on use of organic fertilisers (Regulations on fertilising products etc. of organic origin) is currently being revised, as is the regulation on soil pollution, where reduced background levels of metals have been suggested, notably for Cd, Hg, and Zn (but raised for Pb). The amounts permitted to be used is limited to a maximum application of 4 tonnes/daa/10 years if sludge quality corresponds to Class I regarding metal concentrations (see table 6.3-1), and 2 tonnes/daa/10 years if sludge quality corresponds to Class II. Compared with the background level of trace elements in soil (see table 6.3-1), Zn represents the largest addition when sludge is added to soil, but represents only a four-times higher concentration. When adding 2 tonnes/daa, with a maximum of 800 mg Zn/kg, the increase in soil concentration represents 2 % of the background level (e.g., from 200 mg/kg to 204 mg/kg, assuming 400 tonnes soil/daa). Grain crops contain around 50 mg Zn/kg (or 25 g/daa) and will thus remove approximately 15 % (250 g) of the added Zn during a 10-year period (yield: 500 kg/daa).

Table 6.3-1. Mean concentrations of metals in sewage sludge from Norwegian WWTPs as compiled by SSB for 2018, and regulatory limit values for metal concentrations for sludge applied to agricultural land (Lovdata, 2003).

	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Mean conc. 2018	0.6	17	165	0.3	13	14	391
Regulatory limit (4t/daa)	0.8	60	150	0.6	30	60	400
Regulatory limit (2t/daa) (Blytt and Stang, 2019)	2	100	650	3	50	80	800
Norm, non-polluted soil[9]	1.5	50	100	1	60	60	200

Other antimicrobial agents are monitored less closely. Increasing concern about spreading of pharmaceuticals and other chemicals through the sewage system has led to expanding monitoring schemes, including new compounds in recent analytical campaigns. The most recent inventory of contaminants in Norwegian sewage sludge used samples from 18 WWTPs and included, apart from a wide range of pollutants of environmental concern (including some with antimicrobial properties like triclosan and various phenols), 71 pharmaceuticals and the elements As and Ag (Blytt and Stang, 2019). These monitoring campaigns are costly and far less frequent than the monitoring of metals and are thus conducted only approximately once every 5 years since 1996/1997. Monitoring of organic

[9] SFT 2009 Health-based classification of polluted soil, Report TA2553. Norwegian Pollution Agency

contaminants shows that the concentrations of some compounds with antimicrobial properties, such as phenols and triclosan, are decreasing compared to the previous sampling occasion (2012/2013), while the concentrations of others (such as organotin compounds) have increased during the last 5 years. However, the concentrations were still below the corresponding mean concentrations of the same compounds reported for other Scandinavian countries (Blytt and Stang, 2019). No temporal trends on concentrations of pharmaceuticals in sewage sludge are given this report, as previous inventories have not included such analyses. There is also a lack of data regarding residues of the antibacterial agents most frequently used in human and veterinary medicine in Norway.

6.4 Antimicrobial agents in soil

Heavy metals are elements and thus not degraded. Their fate in soil is, however, influenced by leaching and removal in harvested crops, and by a range of slow chemical processes leading to immobilization. Low rates of leaching and removal normally may lead to slow accumulation, but immobilization processes seem to prevent any net increase in crop uptake (Rivier et al., 2019).

In contrast to metals, pharmaceuticals and other organic pollutants with antimicrobial effects are prone to microbial degradation when entering soil. Biocides, like triclosan and phenols, thus have limited lifetimes in soil, and 90-99% of these are degraded within a growing season after application to soil, even under relatively cool Norwegian conditions (Rivier et al., 2019). For antibiotics, degradation rates in soil vary both between compounds and according to soil conditions, with reported stability ranging from 2.9 to 43.3 days for tetracycline, sulphamethazine, norfloxacin, erythromycin, and chloramphenicol, with the three latter reaching non-detectable residual soil concentrations within 35-50 days (Pan and Chu, 2016).

7 Exposure

7.1 General remarks

Based on the studies and data presented in the previous chapter, this chapter presents an assessment of to which degree the environmental recipients of effluent WW and sludge is exposed to ARB, ARG and RD.

7.2 Exposure to AMR via the effluent water fraction

Effluent water consists mainly of treated WW, as the vast majority of dry matter, microorganisms, and contaminants has been removed. In addition, a small percentage of untreated overflow water from extreme precipitation events is discharged into the same recipients as the treated water. For the largest WWTP in Norway (VEAS), this volume has varied between 0.7-2.6 % of the incoming water volume during the last 5 years (VEAS, 2019).

Effluent water is typically discharged into a deep-water recipient where exposure to AMR primarily concerns local benthic and pelagic species. In semi-contained recipients, like fjords and lakes, the discharge depth may coincide with anoxic layers, preventing transfer to organisms that are part of the human food chain. Diluted effluent water will be transported over long distances with currents, that may eventually lift such diluted AMR elements to surface water layers. When effluents are discharged into marine waters, flocculation and sedimentation of dissolved and suspended matter will occur because high ionic concentrations frequently destabilize dissolved ions, and colloidal and particulate suspensions, causing aggregation and subsequent sedimentation. This phenomenon will also contribute to reducing the spread of AMR from discharged effluents.

7.3 Exposure to AMR via the use of sludge as fertilising material in agriculture

Current regulations on the spread of sludge on soil limit the use to 2 or 4 tonnes/daa/10 years and oblige farmers to plough it in within 18 hours. Cultivation of vegetables, potatoes, etc. is not permitted until 3 years after sludge application (Lovdata, 2003). In agriculture, sewage sludge is almost exclusively used as a fertilising product for cereals and oil seeds. This prevents transfer of soil particles (potentially containing AMR) to edible plant parts (edible parts of grain crops are usually found >40 cm above ground and enclosed in husks until they are harvested). The 3-year period before vegetables can be grown on sludge-amended soil is intended as a barrier against survival and transmission of pathogens to food through direct contact with soil. Long-term survival of bacteria introduced to soil is generally very low (Acea et al., 1988). Thus, both temporal constraints and low amounts of resistance

drivers in soil contribute to limiting possibilities for direct transfer of ARB from sludge to foods. The extent to which ARB survive in soil, maintain their AMR properties, or transfer AMG to indigenous species varies between genes and depends also on soil amendments and soil quality (Thanner et al., 2016). Most knowledge on this concerns AMR spread in animal manures, which require less processing and no hygienisation before spreading, thus potentially retaining more of the initial AMR. The application of manures to soil is also more frequent and uses larger amounts than that of sewage sludge and are partially spread on growing plants (fodder) and at the soil surface. These aspects indicate a higher exposure to AMR from manure than from sludge. However, the volumes of antibiotics used in human medicine and animal husbandry have a ratio of 85:15 in Norway (Steinbakk et al., 2014), indicating that the relative contribution of manure to AMR exposure may be lower in Norway than in most other countries.

7.4 Exposure to antimicrobial agents via the use of sludge as fertilising material in agriculture or as component of produced soil

This section is partially based on data in chapter 4.2 and 4.3 and data presented in (Rutgersson et al., 2020).

The strict regulations on the use of sewage sludge in agriculture is likely to prevent any exposure to antimicrobial agents through ingestion of food. Soils are generally efficient at immobilizing organic molecules, as they tend to adsorb to humic materials (compounds characterized by medium to high K_{ow}-values) and clay (charged compounds). The octanol/water partition coefficient is defined as K_{ow}. This limits mass flow of organics towards roots, and thus plant exposure. Furthermore, plants generally have strong barriers against absorption of organic molecules from soil. As an example, triclosan (log K_{ow}=4.8) added to soil at 4 mg/kg soil resulted in undetectable leaf concentrations in barley (Macherius et al., 2012). Organic molecules are also metabolized and degraded in soils, with half-lives of most antimicrobial agents being comparatively low. A notable exception here are heavy metals, which do not degrade; but all soils contain heavy metals of natural origin, as do plants that grow on these soils. Humans and animals have an intrinsic tolerance to low levels of heavy metals, and the incremental increase within a Norwegian diet due to agricultural use of sewage sludge is insignificant.

Sewage sludge is also used for production of soil for green areas, like roadside slopes and urban decorative plantations (not lawns). As sludge is nutrient rich, the sludge content of such soils is <5 %. When urban plantations are part of public parks, children may, theoretically, come in contact with sludge-based soils and ingest small amounts.

7.5 Exposure to AMR in WWTP workers and farmers

Irrespective of the fate and use of sewage sludge, workers at the WWTPs will be exposed to microorganisms in the sewage and the resulting sludge, and such exposure can be significant (STAMI, 2016). During transport to farms and farmers' exposure (or exposure to workers at soil production companies using sludge for production of soil) are direct and unique exposure risks due to the current use of sludge as a soil amendment. The working group has not been able to find information about the extent or the impact of such exposure on the workers/farmers that are in direct contact with WW and/or sludge.

8 The assessment of the probability for AMR development via effluent wastewater and applied sludge

8.1 Approach

The ultimate health risk related to AMR is failure in the treatment of infectious diseases. The emerging AMR threat may bring humanity back to the pre-antibiotic age, which will not only hamper our possibilities to treat specific infections, but it will also considerably hinder many modern medical-treatment procedures that currently depend on antimicrobials for prevention of post-treatment infections. As commented in chapter 3, due to too many uncertainties associated with the release of ARB, ARG and RDs from WWTPs into different environmental compartments and treatment failures in human (and veterinary) medicine, it is impossible to make a full risk assessment of the issues raised in the assignment from NFSA and NEA. Rather than present a full risk characterization, in this chapter we summarize our findings into a "Characterization of probability for development and dissemination of AMR via effluent wastewater and applied sludge". We have not been able to transform our assessments into a quantitative probability characterization.

Some of the referenced literature in the previous chapters presents data from countries that have a much higher use of different antimicrobial agents for human, animal, and environmental applications. Parallel to this, the endemic levels of AMR in these countries are generally higher than in Norway. It is therefore important to note that this probability characterization takes into account some of special characteristics of Norwegian society: the general level of consumption of antimicrobials is lower than in most other countries; we use a relatively high amount of beta-lactamase sensitive penicillins, and we use low levels of 3rd- and 4th-generation cephalosporins and fluoroquinolones (EMA, 2017). We also have good infrastructures for sanitation. In 2016, 62 % of the population in Norway were connected to a WWTP that uses approved biological or chemical treatment processes, and the coverage was especially high in urban areas. In only a few places and remote areas is untreated WW discharged directly into the environment (Statistisk sentralbyrå, 2018). However, still more than 30 % of the Norwegian population are not connected to sewage systems and treatment which cannot fulfil the requirements. This brings about uncontrolled discharge of wastewater likely carrying AMR.

There is also an excess of water in Norway; more than 98 % of the population have drinking water of satisfactory quality, and every individual uses an average of 180 L of water pr. day for household purposes (Statistisk sentralbyrå, 2018). Most drinking water sources in Norway are surface waters, such as lakes and rivers. These are more vulnerable than ground water to exposure to faecal and chemical contamination. Good drinking water quality is ensured by

strict legislation for control of activity in the watersheds and the drinking water plants, under the control of the NFSA. However, leakage of Norwegian drinking water distribution systems is enormously high and about 30 % on average and far higher than as for example in Sweden (15 %) and Denmark (6 %). Leakages always bring about a high risk for intrusion of surface or even wastewater, which with high probability contains AMR.

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, via which route microbiota of animal origin may end up in the public sewage systems. Animals sent to slaughterhouses are healthy, have not received antimicrobial treatment before slaughter, and only stay at the slaughterhouses for a few hours. A similar situation occurs in the dairy industry: milk from animals receiving antimicrobial treatment is not sent to the dairy. Notably, the use of antibiotics in production animals in Norway is very low, and only consists of about 15 % of the total use of antibiotics in the country. Faecal microbiota from dogs and horses in urban areas may be discharged into the sewage system, especially in association with overflow from heavy rainfall etc. Altogether, the contribution from animal host microbiota regarding development and dissemination of AMR in WWT processes is considered to be low, relative to the contribution from human activity.

This indicates that the most prominent routes for dissemination of AMR are, as illustrated in Figure 1-1, as follows: discharge of human faecal microbiota into sewage; treatment procedures at WWTPs; release of WW effluents and application of sludge onto agricultural land; possible recycling into the food chains, and then consumption of food. Some observations indicate that the aquatic environment is a particularly important reservoir of novel antibiotic resistance determinants (Aubron et al., 2005; Cattoir et al., 2008). In some areas, the relative abundance of such resistance determinants in effluents has been observed to be considerably higher than in pristine natural water sources, suggesting that WW represents a source of ARG contamination to the receiving water systems (Berglund et al., 2015; Brown et al., 2019; Czekalski et al., 2015; LaPara et al., 2011; Shao et al., 2018).

It is inevitable that humans are exposed to environmental microbial community reservoirs. This may be associated with risk, but it is important to take into consideration that exposure to environmental microbial community may also have benefits.

8.2 Probability for direct discharge of ARB into effluent wastewater and applied sludge

Chapter 6.2 describes the fate and survival of ARB in WWTPs. In this chapter, several studies are referenced with contradictory results regarding the levels of AMR in effluent WW and sludge fractions discharged from WWTP. Chapter 6.4 specifically discusses the content of ARB in hospital WW. An overall assessment is difficult due to the complexity of WW systems and the lack of harmonized methods and protocols that enable comparison of data from different systems. However, there are no indications that there is a significant selection for

ARB in WWTPs under European conditions; thus, this conclusion also applies to the Norwegian situation. Although some studies indicated a slight increase in the fraction of ARB, the absolute reduction over treatment is significant and removals of between 99 % to 99.9 % of faecal indicator bacteria are generally achieved in Scandinavia via secondary treatment, including biological and physico-chemical treatment steps. This is consistent with a comprehensive antibiotic-resistance screening of 4 028 *E. coli* isolates from a WWTP in Sweden, which provided no support for selection of AMR when aggregated data from multiple samplings was analysed. Additionally, there was no increase in the MAR index (Flach et al., 2018). Regarding removal of resistant enterococci in WW treatment, some studies from Portugal and Poland indicated positive selection for ciprofloxacin-resistant enterococci through the WWTP (Ferreira da Silva et al., 2006; Luczkiewicz et al., 2010).

Effluent wastewater is released into deep-water recipients, and there are many mechanisms (physical, mechanical, and chemical) that limit the probability that ARB of faecal origin will be transferred to food-production chains. Results from the few published studies indicate that WWTP effluents probably contribute very little to the total exposure of organisms in aquatic and marine environments to AMR. However, a certain imprint of AMR in recipient water bodies compared to pristine waters is unavoidable.

Effluent wastewater is often released into deep-water recipients or rivers, and there are many mechanisms (physical, mechanical, and chemical) that limit the probability that ARB of faecal origin will be transferred to food-production chains. Results from the few published studies indicate that WWTP effluents contribute little to the total exposure of organisms in aquatic and marine environments to AMR. However, a certain imprint of AMR in recipient water bodies compared to pristine waters is unavoidable.

During WWT, bacteria largely adhere to particles that are aggregated and precipitated to form a solid sludge. The mandatory hygienisation of sludge inactivates a large proportion of these bacteria, notably all thermosensitive faecal bacteria. The resulting hygienised sludge is still rich in bacteria, and some will be carriers of ARGs. The current Norwegian regulations on use of sludge on soil contribute to ensuring that contamination of food with ARB and ARG from sludge is minimised. However, soils do contain a pool of both natural and sludge-derived AMR. The contribution of sludge to this AMR pool is probably low and temporally limited to a period immediately after soil amendment with sludge. The persistence and maintenance of AMR in soil is largely unknown, particularly for Norwegian conditions.

8.3 Probability for selection and increased abundance of ARB due to presence of ARG and antimicrobial residues in effluent wastewater and applied sludge

Chapter 6.3 describes the occurrence and fate of ARG in WWTPs, effluent WW, and applied sludge. As emphasized, a general interpretation of all data is problematic due to the

complexity of WW systems in general, the limited information on expression patterns and origins of ARGs on site throughout the treatment process, and the lack of harmonized methods and protocols that would enable comparison of different WWT systems (Karkman et al., 2018).

In their study of sewage-impacted environments in different places around the world, Karkman et al. showed that increased ARG levels can often be explained by the presence of faecal residues (Karkman et al., 2019). They were nevertheless able to detect true hot spots for ARG selection in sediments receiving exceptionally high levels of antibiotics from manufacturing industries. In all other studied environments, the ARG abundance correlated strongly with faecal pollution. They concluded that their study did not support the opinion that major selection for AMR occurs in WWTPs or in effluent-receiving environments.

Similar considerations have been done for effluent WW as for sludge, as discussed in chapter 5.2.5 and supported by the studies of (Bengtsson-Palme et al., 2016) and (Karkman et al., 2019) and the review by (Li et al., 2019); overall, WW treatment tends to reduce the content of ARGs in the effluent.

Sewage sludge in Norway is regularly assessed for numerous contaminants, including pharmaceuticals, biocides, and other chemicals. The stability of these antimicrobial agents in sludge is described in chapter 4.2.3. Exposure to antimicrobial agents is regarded as the most important driver for development and dissemination for AMR in microorganisms. The traditional perception is that exposure to one antimicrobial agent subsequently results in development of resistance to this particular agent. However, due to phenomena such as cross- and co-resistance, the dynamics of this development are more complex and are also influenced by many other interconnected driving factors. Some studies have shown that exposure of *E. coli* to an antimicrobial such as penicillin promotes resistance to several unrelated antimicrobial agents (Grønvold et al., 2010; Grønvold et al., 2011), probably through the activation of the bacterial SOS-response, which, again, activates resistance-transfer mechanisms. In contrast, a more recent study demonstrated that low concentrations of Cu and Zn reduce the conjugation frequency of resistance plasmids in ESC-resistant *E. coli* (Buberg et al., 2020). However, neither of these studies were conducted in a WW/sludge environment.

Hughes and Andersson emphasize that there are still too little data available for quantifying either the magnitude or direction of transmission of ARG or resistant pathogens between humans and environmental reservoirs, such as soil and water (Hughes and Andersson, 2017). In the summary of chapter 5, it is emphasized that based on current literature, it is impossible to draw any conclusions on potential selection of ARGs in WWT processes. The relative impact of antimicrobial residues in this picture is even more difficult to evaluate.

A recent study by Rutgersson et al. showed that long-term application of sewage sludge to farmland in Sweden resulted in only minor changes in the composition of the soil bacterial community (Rutgersson et al., 2020). The results of their comprehensive study were summarized as follows: "We could not find evidence, neither on a short- nor on a long-term

scale, for accumulation of antibiotics or an enrichment of ARGs or ARBs in soil amended with digested and stored sewage sludge in doses up to 12 tonnes per hectare every four years. No evidence of co-selection via metals or biocides measured as abundance of metal-resistance genes (MRGs) and biocide-resistance genes (BRGs) could be found. Additionally, very few alterations in the microbial community structure were observed due to the sludge application."

Some studies from recent years reached different conclusions. Both Urra et al. and Chen et al. observed that application of sludge increased the abundance of ARG in soil at their study sites in Spain and China, respectively (Urra et al., 2019) and (Chen et al., 2016). The amount of sludge applied to their sampling fields was also higher than the amounts used in the study of Rutgersson et al. in Sweden. Thus, the occurrence rates of ARB and ARG in sludge-amended soil may be related to the dose of antimicrobials. In this respect, the Swedish situation is similar to the Norwegian situation regarding antimicrobial usage and problems with AMR.

8.4 Summary of probability characterization

This opinion presents, compiles and discusses available information regarding the probabilities for development of AMR through effluent WW and applied sludge, summarized in Table 8.4-2, using the definitions based upon the VKM's Terminology Guidance (VKM, 2018).

Table 8.4-1. Scale for probability, in five steps. The explanation of the scale will vary between subject and subject area for assessments. (VKM, 2018).

	Subjective probability range
Very likely	90-100 %
Likely	66-90 %
As likely as not	33-66 %
Unlikely	10-33 %
Very unlikely	0-10 %

Table. 8.4-2. Probabilities **for the development of AMR** in bacteria through WW, effluent WW and applied sludge. The probability estimation was partly based on the available data, but also based on expert opinions as the available data were insufficient

Probability of development of resistance in microorganisms	**Very likely**	**Likely**	**As likely as not**	**Unlikely**	**Very unlikely**
Wastewater treatment plants (WWTPs)					
Direct selection of ARGs and ARBs in municipal WWTPs			X[A]		
Direct selection of ARGs and ARBs in WWTPs from hospitals and pharmaceutical industries		X[B]			
Selection of ARB due to co-selection by antibiotics, antifungal, and/or biocides and metals in WWTPs	X[C]				
Selection of ARB due to HGT of ARGs between pathogenic faecal bacteria and indigenous environmental bacteria in WWTPs	X[D]				
WW discharges: Effluent water fraction (E)					
Increased abundance of ARBs/ARGs after WWT				X[E]	
Accumulation of ARBs and ARGs in receiving waterbodies				X[F]	
WW discharges: Applied sludge fraction (S)					
Increased abundance of ARBs/ARGs after sludge treatment			X[G]		
Selection of ARB due to co-selection by antibiotics, antifungals, and/or biocides and metals in soil				X[H]	
Accumulation of ARBs and ARGs in fertilized soil				X[I]	
Increased levels of ARB in food due to transmission of AMR from sludge to agriculture				X[J]	

[A]ARB that dominate the resistant fraction of the sewage microbiota will reach the WWTP, but at lower levels in some infrastructures due to local fermentation and competition.

[B] Investigations related to emergence, occurrence, and dissemination of ARB and ARG in hospital WW are very diverse. In general, bacterial AMR profiles mirror the antimicrobial consumption at that location, which usually is highest in hospitals. However, the relative contribution of hospital effluents may be low in terms of dissemination of ARB to the WWTP.

[C] Antimicrobial residues will reach the WWTP, but at varying concentrations depending on dilution. There is a need to map each of these resistance drivers individually and also to analyse the interactions between the various groups of antimicrobial compounds.

[D] The sewage system is regarded as a potential hot spot for HGT events between different microorganisms.

[E] In summary, there is no significant enrichment of ARB in WWTPs under European conditions. Some studies indicate a slight increase in the fraction of ARB. However, the absolute reduction during treatment is significant and removals between 99 % to 99.9 % of faecal indicator bacteria are generally achieved in Scandinavia by secondary treatments, including biological and physico-chemical treatment steps.

[F] Increased levels of AMR can often be explained by the presence of faecal residues. Recent studies conclude that there is no major selection for AMR in effluent-receiving environments, rather the opposite; overall, WW effluent tends to have a lower content of ARGs. However, transfer of ARG between gram-positive bacteria within an aquatic environment is regarded as likely.

[G] There are indications that conventional sludge treatment methods are not particularly efficient at ARG removal.

[H] An extensive study from Sweden on effects of long-term application of sewage sludge to farmland found no evidence of co-selection via metals or biocides, as measured as abundance of metal-resistance genes and biocide-resistance genes.

[I] The most relevant study is from Sweden, in which long-term application of sewage sludge to farmland was shown to result in only minor changes to the composition of the soil bacterial community. No evidence could be found for enrichment of ARB or ARG in soil amended with digested and stored sewage.

[J] The bacterial concentration in sludge may be much higher than in untreated WW (pr. gram), depending on treatment methods applied. Current regulations on use of sludge on soil and farmers' practices, and the fact that survival of microorganisms from other reservoirs that are introduced to soil is low, mean that the probability of direct transfer of resistant bacteria from sludge to foods is also low.

9 Needs for monitoring and possible indicators

In the ToR, NFSA and NEA request VKM to identify indicators that can be used for monitoring and control of resistance driving chemicals (antibiotics, antifungal agents, heavy metals, disinfectant agents etc.) in wastewater effluent and sludge destined for use as fertilising product. Norway already has two surveillance programmes for monitoring the use of antimicrobials and AMR in humans and in animals and food: NORM and NORM-VET, respectively.

As noted in chapters 4.2.4 and 6.2.1 – 6.2.4, systematic measuring of RDs in WW is absent in Norway and only limited data on the presence and concentrations some RDs are available. Norwegian WWTPs monitor some chemicals and elements in in-coming WW, effluents, and sludge, mainly as a means of verifying the performance of the treatment methods used. The most closely monitored parameter is PTMs, and studies show that the WWTPs remove a large proportion of most of these heavy metals. This demonstrates that treated WW for example represents a very minor fraction of the anthropogenic load on coastal waters for PTM. Norsk Vann has also reported on the content of organic contaminants in sludge from WWTPs in 2017/18 and 2012/13 (Blytt and Stang, 2019).

The relative importance of the variety of RDs, the dynamics of the interactions between RDs and microbial populations and the dose-response patterns regarding AMR development are still far from understood. Based on the identified uncertainties (chapter 11) and knowledge gaps (chapter 13), we suggest that monitoring of AMR, rather than RDs, in WW effluent and sewage will be most relevant to identify and understand the introduction of AMR into the environment and food-production systems. Systematic collection of such data over time and space will assist in assessing the exposure levels to bacteria and their resistance characteristics. Such understanding is necessary to assess risks to human-, animal-, and environmental health, - in a One Health perspective.

A lot of expertise, capacity, routine methods, standard procedures, and infrastructure for AMR monitoring have been developed, established, and coordinated through the NORM and NORM-VET programmes. Should a new programme for surveillance of AMR in effluent WW, sludge, and sludge-amended soil be established (e.g., "NORM-ECO"), it would be advantageous to leverage from the experiences already obtained with NORM and NORM-VET.

It should be emphasized that as NORM and NORM-VET are programmes for monitoring the situation regarding antimicrobial usage and AMR; the focus of "NORM-ECO" should be on monitoring and not control as well. There is still relatively little knowledge on the presence and dynamics of AMR in non-clinical compartments compared to hospital and other clinical

settings, and a single parameter that would trigger an immediate response from NFAS or NEA has not yet been identified.

NORM and NORM-VET use culture-based methods to screen for AMR in faecal indicator bacteria, *E. coli,* and enterococci. Several studies support the choice of *E. coli* as a target, as *E. coli* is very well established as a widely monitored target for faecal pollution (Blaak et al., 2015; Hiller et al., 2019; Liu et al., 2015). Berendonk et al. suggest that *Klebsiella* and faecal enterococci would be appropriate alternative targets for monitoring (Berendonk et al., 2015). In Norway, Paulshus et al. concluded in their study of untreated WW from a hospital and a residential area that measuring levels of AMR in *E. coli* from WW samples can indicate the level of AMR in the corresponding human population, and can be used as an early warning system for changes in resistance patterns in society (Paulshus et al., 2019b).
As *E. coli* may not be a suitable representative for bacteria that have aquatic and/or soil environments as their natural reservoirs, *Pseudomonas* spp. and *Aeromonas* spp. may be relevant choices (Santoro et al., 2015; Vaz-Moreira et al., 2016). Resistance-determinant targets can be differentiated into those of direct clinical concern and those for use as indicators. In a surveillance programme, the choice of relevant resistance determinants should be coordinated with NORM and NORM-VET. In a recent review, Hiller et al. suggested that the following indicator ARGs could be used to monitor for AMR: resistance genes of broad-spectrum antibiotics, *sul1, sul2*, and *tet* genes *(tetA, tetB, tetO* and *tetW*), as well as resistance genes against antibiotics of last resort, vanA and blaVIM (Hiller et al., 2019).

Culture-based methods for identification of bacterial species and AMR characteristics have their strengths and weaknesses. Gene sequence-based methods are increasingly replacing these, but must be chosen carefully depending on various factors, including purpose, costs, and time. Metagenomic methods are still in their infancy regarding studies of WW and sewage, and highly advanced competence and infrastructure are prerequisites for their use. Hendriksen et al. used a metagenomic approach in a large, multicentre study of untreated sewage, and concluded that "metagenomic analysis of sewage is an ethically acceptable and economically feasible approach for continuous global surveillance and prediction of AMR" (Hendriksen et al., 2019).

Huijbers et al. presented "a conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance" (Huijbers et al., 2019). The authors emphasized that the design of a surveillance programme must be based on clear and consistent definitions of surveillance objectives. They present the characteristics of five different objectives for environmental surveillance that could be addressed either separately or in parallel.

10 Suggested mitigation measures

10.1 Education/Public awareness campaigns

Generally, all measures that reduce the use and release of antibiotics, antifungals, disinfectants, and other resistance-driving substances, such as heavy medals, will decrease the spread of AMR. This is especially by limiting AMR received at WWTP. Data compiled by Meyer et al. on the use of antibiotics in DDD in 11 European countries, showed that use of antibiotics was lowest in Denmark, Hungary, Norway, and Sweden (Meyer et al., 2013). As discussed in chapter 6.5, antibiotics administered to humans in Norway are about 2/3 of the amounts administered in Germany (6.5 per inhabitant per year compared with 9.0 per inhabitant per year). Restricting the use of antibiotics even further could decrease release to the environment, but it is unclear how far antibiotic use could be restricted without increasing the risk for patients.

About 91% of antibiotics consumed for human health in Norway is by outpatients. This offers further options to tackle the discharge of antibiotics to the environment. Nationwide campaigns aimed at improving use of antibiotics have led to a reduction in outpatient use (Huttner et al., 2013; Meyer et al., 2013), demonstrating that educational campaigns can result in significant effects. Therefore, further educational campaigns should be considered to increase public awareness and understanding about environmental and health risks associated with the use and careless discard of antibiotics. Such campaigns should be followed by behavioural and sociological studies, and communication science should study the effects achieved alongside the campaigns.

Ideally, campaigns focusing on the use and environmental relevance of antibiotics should simultaneously address the environmental implications of other agents, such as chemicals used in households. To improve responsible handling of antibiotics and other agents, user-friendly take-back systems must be made readily available to the population.

10.2 Source separation / Collection of urine from patients

Generally, administered antibiotics are not completely metabolized, and therefore they are found in WW. This is why WW is considered a hot spot for development of AMR. As mentioned in chapter **Feil! Fant ikke referansekilden.**, there is no systematic monitoring of antimicrobial residues in WW in Norway. As discussed in chapter 6.5, Norway, as other Scandinavian countries, has a relatively low consumption of antibiotics for human health in comparison with other European countries. Interestingly, while the use of antibiotics for animals in total is much higher than for humans in other countries, the converse is true in Norway. Here, about 85 % of antibiotics are used for humans, and, of these, the vast majority (91 %) are for outpatients, including in nursing homes. This means that about 70 % of antibiotics used in Norway is used by humans who are not inpatients in hospitals.

Assuming that the proportion of antibiotics not metabolized and excreted is approximately the same for humans and animals, 70 % of all antibiotics discharged to the environment originates from humans who are not in hospital.

Although WWTP are suspected as being hot spots for the development of AMR, convincing evidence that this is the case is currently lacking. Indeed, the concentration of bacteria decreases from influent to effluent in the WWTP. In addition, studies that take into account uncertainties of the analytical methods and that use sufficient samples for reliable statistical analysis (Pallares-Vega et al., 2019), show that the concentration of ARG decreases significantly from influent to effluent and, to the same extent, for bacteria in general.

This means that the highest concentrations of antibiotics, ARB, and ARG occur in the raw sewage after excretion by the people to whom they are administered. Consequently, for Norway, discharge of 70 % of antibiotics, and probably ARB and ARG of roughly the same percentage, to the environment could be stopped if the urine and faeces of people taking antibiotics was collected rather than being flushed down the toilet.

Should only urine be collected separately from people taking antibiotics, the influx into the sewage system of ARB and ARG would be considerably reduced. In a recent project funded by the German Federal Environmental Foundation, separation of urine from patients who had been administered X-ray contrast was successfully trialled (Thöne, 2018). In cooperation with two hospitals and two doctors' practices, a concept was established to separate urine from these patients using urinal bags. These bags are commercially available, for example for outdoor use, and contain a superabsorber that binds the urine and solidifies. After being provided with information by the doctors, the patients received a package that contained an information leaflet and four pocket urinal bags. The project was supported by media campaigns. Using this approach, urine was collected in a hygienically safe way, and could be disposed of with the garbage for incineration in waste incineration plants. Due to close cooperation and a well-designed communication strategy, up to 87 % of patients used the pocket urinal bags. The additional costs for the source collection of urine were estimated to be approximately 6 € per investigation.

A somewhat similar system for source separation of urine from patients in Norway taking antibiotics might be relevant for some specific antibiotics, and could be worthwhile to consider more closely. This simple front-end approach might mitigate the dissemination of AMR at far lower costs than technical end-of-pipe measures.

10.3 Source separation of heavy metals

There is increasing awareness regarding prevention of toxic chemicals ending up in the WW system. Return and collection points for toxic chemicals and other environmentally damaging compounds have been established locally in each municipality in many countries, including in the EU. The resulting metal contamination of soil and water is thus decreasing, and a 25-year trend regarding the metal content of Norwegian sewage sludge show a similar

reduction for the most relevant metals. Only very minor amounts of metals reach soil and water through sewage effluent and sludge, and their contribution towards driving resistance in bacteria in soil and water is likely to be very low. Thus, there is little incentive to impose measures to reduce the amounts of metals reaching soil and water through effluent water and sludge.

10.4 Rehabilitation of sewage networks and drinking water distribution systems

As discussed in chapter 10.2, the concentrations of antibiotics in sewage are the highest at the influent to the sewage treatment plant, i. e. in the sewage system. FHI (Folkehelseinstituttet) concluded that about 33 % of Norway's population are connected to sewage systems and treatment, which do not fulfil the requirements (FHI, 2018). Clogging of sewage systems happens often. In 2016 there were about 53 cloggings per 1,000 km of sewage system, which was an increase of about 10 % compared to 2015. Each such clogging results in an uncontrolled, often late discovered overflow to the aquatic environment and thus an uncontrolled discharge of AMR.

At the same time, the drinking water distribution system needs continuous maintenance, as can be seen from the leakage which is, on average, about 30 %. In some areas, leakages are above 50 %. For comparison, in neighbouring countries leakages are much smaller, as e. g. about 15 % in Sweden and about 6 % in Denmark. Each leakage also poses a relative high risk for intrusion of contaminated water from the surrounding of the leak into the drinking water system. This might also contain AMR.

Concluding, rehabilitation of the sewage as well as the drinking water distribution system will considerably lower the risk from AMR in wastewater.

10.5 Upgrading sewage treatment plants in general

Generally, the literature indicates that AMR removal (ARB and ARG) improves with increasing levels of WWT, although today's WWTPs are not designed for removal of trace contaminants including ARB and ARG (Krzeminski and Popowska 2020). The more advanced the treatment is, the lower is the likelihood of dissemination of AMR with treated sewage discharge. However, the efforts to be taken are unfortunately largely exponential with the effect to be achieved.

10.6 Advanced sewage treatment (4th-stage treatment)

To minimize emissions that can contribute to the increase of AMR in the aquatic environment, antibiotics as well as ARBs and ARGs should be removed as much as possible during WWT. As shown by Pallares-Vega et al. and discussed in chapter 5, WWT decreases the concentration of bacteria (Pallares-Vega et al., 2019). However, the concentration of ARB

and ARG does not decrease or increase any differently to that of other bacteria. As summarized by Hiller et al., additional removal of ARB and ARG requires further treatment (Hiller et al., 2019). This can be achieved by advanced sewage treatment processes, as discussed in chapter 4.4.3.5. As with primary, secondary, and tertiary treatment, increased removal of ARB and ARG in this way will be accompanied by the removal of other bacteria.

Generally, three different process principles or mechanisms can be applied. The first principle, size exclusion, uses media with pores that are smaller than the bacteria, and can thereby retain them, while the water passes through (Breazeal et al., 2013; Böckelmann et al., 2009). For ARG, this requires ultrafiltration membranes. However, provided that removal of bacteria is intended, membranes with larger pores (i.e., microfiltration membranes) might also be adequate, as the formation of cake layers can significantly improve overall performance. Thus, use of MBR in secondary treatment seems to be promising for improving removal of ARB and ARG (Munir et al., 2011; Park et al., 2011; Zelante et al., 2013; Zhang et al., 2015b). One disadvantage, however, is that MBR are very energy intensive.

Using membranes as final treatment (4th stage) could be advantageous regarding energy consumption, as particles and organic matter will have been considerably reduced in the preceding steps. Krzeminski et al. showed that ultrafiltration membranes can also remove ARG (Krzeminski et al., 2020). Their investigations used water that did not contain any particles. As particles form a cake layer on the membrane and DNA can adsorb onto the particles, removal of ARG could also be achievable using microfiltration membranes.

The second process principle of relevance is chemical disinfection, or oxidation, using chemicals. Ozone, which also has germicidal effects, is often applied in the 4th stage of WWT for the oxidation of trace organic compounds. However, the disinfection efficacy of ozone is limited by a number of compounds in the WW matrix that result in a rapid depletion of ozone (Czekalski et al. 2016; Pak et al. 2016; Zucker et al. 2015). Elevated removal efficiencies require higher doses, which are, however, not feasible (Oh et al., 2014). In contrast to ARB, removal of ARG is even more limited in WWT (Alexander et al. 2016; Czekalski et al. 2016) as very high ozone doses are needed (Zhuang et al. 2015). However, such high doses are uncommon in WWT, because ozonation of WW is mainly intended for removal of trace organic chemicals. As the exact mode of action of ozone on microbial cells is currently not completely understood, further research is needed in this field.

As with ozone as oxidative treatment, chlorine also acts on ARB as it does on bacteria in general. In the literature, studies that focus on the effects of chlorination on ARB have confirmed experiences from disinfection of non-resistant bacteria. However, incomplete inactivation of ARB, as well as re-growth of ARB, was observed (Oh et al. 2014; Huang et al. 2011; Munir et al. 2011). Low chlorine doses seem to be ineffective, which is not unexpected as chlorine is consumed by particles and organic substances in the WW matrix. For ARG removal, studies showed only small effects from chlorine treatment (Yang et al. 2016; Zhang et al. 2015; Yuan et al. 2015; Fahrenfeld et al. 2013).

Of the oxidative treatment processes, ozonation is preferable to chlorination, as chlorine results in the production of chlorinated organic substances that will then be released and pollute the environment, which is generally not considered as particularly problematic for ozone. However, a multitude of reaction products might result from the reaction of ozone with unknown substances in the WW matrix. For both ozone and chlorine, or for other oxidants, it must be realised that these chemicals pose stresses on the microorganisms in the WW matrix. Thus, if they are not applied in such a way that all microorganisms are inactivated (which will require extremely high doses), their application might even favour the development of AMR, as outlined in the chapter **Feil! Fant ikke referansekilden.**.

The third process principle is physical inactivation of bacteria, including ARB, using UV light. By using UV disinfection, the total abundances of ARB can be reduced at typical UV fluences used for water disinfection (Guo et al. 2013; Zhang et al. 2015; McKinney et al 2012). However, in treated WW, there are still high concentrations of particles to which bacteria are attached and to which extracellular DNA is adsorbed and could shade the targets from the UV radiation. Thus, extremely high dosages, and thus energy, are needed to achieve effective inactivation.

10.7 Treatment of hospital wastewater

The concentration of antibiotics in WW from hospitals in Norway can be estimated as being about ten times as high as in sewage from households. However, on average, the total load of antibiotics discharged from hospitals in Norway is only about 10 % of the total load that originates from diffuse discharge from human use of antibiotics. With this in mind, whether separate treatment of hospital WW would be of value, both from an efficiency perspective and from an economics point of view, should be considered carefully. The question to be addressed is the extent to which WW from hospitals contributes to the total amount of sewage transported to the WWTP.

Separate treatment of hospital effluent is recommended whenever there is no public sewage treatment, or when the WW from the hospital contributes more than 20 % to the total sewage flow rate. In the latter case, the mixture of hospital WW and municipal sewage will contain more than double the amount of antibiotics than "normal" sewage. Discharge of untreated WW into the environment must be avoided.

As outlined by Prüss-Üstün and Townend, WHO recommends that for onsite treatment of hospital WW, the following treatment steps should be included: mechanical pre-treatment (primary treatment), biological treatment (secondary treatment), and tertiary treatment (filtration or maturation pond), to minimise filterable substances to below 10 mg/L concentrations, and, finally, disinfection of effluent using chlorine or UV (Prüss-Üstün and Townend, 1999). Sludges from such plants/units need proper digestion to achieve appropriate levels of hygienisation. Alternatively, the sludges can be dried in sludge beds for subsequent combustion.

Very specific WW from hospitals, such as, for example, effluents from dialysis devices, effluents from haematology labs containing ethidium bromide, or effluents from nuclear medicine, should be collected separately and treated in special treatment units for hazardous waste. Sewage from hospitals, including excrement from patients and staff, grey water from the kitchen area, showers, etc. can be considered being treated separately from other sewage as it might contain hazardous substances or microorganisms, including antibiotics, ARB, and ARG.

Generally, treatment of sewage from hospitals includes the same treatment steps as typical treatment of municipal wastewater. However, WW from hospitals may require further treatment if the municipal WWTP does not include sufficient processes. For example, if the municipal WWTP includes only primary and secondary treatment, the hospital WW should include tertiary treatment. If the municipal WWTP also includes tertiary treatment, then the use of an advanced treatment of hospital WW could be considered, such as ozonation, activated carbon filtration, or membrane filtration.

With respect to ARB, all processes that remove or inactivate bacteria and particles are relevant. This particularly includes membrane processes, as a mean pore size of 0.2 µm is sufficient to retain bacteria. Membrane processes and MBR are advantageous, not only due to their high treatment performance, but also because of their compact design. Finally, there must be a disinfection step for both the treated water and sludges to guarantee hygienisation and to limit the spread of bacteria and resistance.

10.8 Considering AMR in treated sewage sludge

As discussed, all matter removed from the sewage ends up in the sludge. Today's sludge treatment is designed for the inactivation of microorganisms that pose a potential health risk. However, as for the wastewater treatment, sewage sludge treatment is not designed for the inactivation of antimicrobial resistance. For the future, consequently, a closer investigation of the impact of sludge treatment methods on AMR, and the development of effective technologies, should be considered.

10.9 Rethinking the paradigm of sensitive recipients

The current requirements for sewage treatment are currently based on the concept of the sensitivity of the recipient, i.e., on a good natural status of the environment receiving the discharge. This concept was developed decades ago. However, in defining the sensitivity of the recipient, the discharge of nutrients is the measuring stick, and whether the recipient is already loaded with nutrients from different sources. As outlined in chapter 4.4.3.5, the currently ongoing implementation of quaternary treatment in Central Europe is also based on that approach. Areas that are already being stressed by contaminants, should be relieved from receiving more contaminants.

For Norway, with its huge water resources, a different approach might be considered. WW from less densely populated areas receives less treatment, as these areas are usually characterized as being less sensitive. However, AMR is not degraded in the same huge volumes as are nutrients, and AMR, as with most trace contaminants, will accumulate or even develop further. Therefore, it should be considered whether further treatment might be more relevant when recipient waterbodies are in less polluted (sensitive) areas than in areas where nutrient removal must have a very high priority.

11 Uncertainties

The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions identified in all the previous steps. EFSA recommends that assessments identify areas of uncertainties and state clearly their subsequent impact on the overall assessment outcome for the purpose of clarity and transparency in risk-assessment processes. Additionally, this is critical in the subsequent selection of risk-management options (EFSA Scientific Committee, 2018).

Discrimination between uncertainty and variability is important in the subsequent selection of risk management options. Biological variation includes, for instance, the differences in resistance levels that exist in microbiological populations over time, and between hosts and environments, including random fluctuations (FAO, 1999).

In this assessment, a number of uncertainties have been identified related to our understanding of the probability of development of AMR, and the dissemination of AMR from WW. Many of these uncertainties are due to data gaps and the lack of a quantitative framework. This has resulted in a high number of point-prevalence studies, with little coherence between them, and a lack of being representative regarding time and geography.

Some sources of uncertainties identified are:

- Insufficient knowledge on the consequences to human/animals following exposure to residues of antimicrobial agents in the environment.
- Uncertainty on the potential for survival and establishment of ARB in the environment and any subsequent effects on the microbiological balance in the ecosystem.
- A lack of understanding of the genetic interactions and spread that occur in environmental bacteria.
- A lack of knowledge on the ecological roles of antimicrobial agents in the natural environment, in particular the effects at sub-inhibitory levels.
- AMR is an evolving situation; many of those factors that may promote/reduce the transmission of ARB, and their corresponding gene determinants, have not yet been identified.
- The lack of being able to trace back HGT events to enable identification of the conditions and circumstances that gave rise to the specific event.
- Uncertainty in measuring use of antimicrobials; all antibiotics purchased in Norway are included, but if they are bought abroad, they will not be included.
- Data about sewage treatment is limited, with very few relevant, long term-studies (Rutgersson et al., 2020).
- WWTPs and sludge treatment facilities are quite heterogenic when it comes to technique and volume. There is also large variations within a WW system with flowrates and type of influents. This will affect the results of the studies.

12 Conclusions (with answers to the terms of reference)

1. **Describe wastewater treatment methods used in Norway today and how these methods affect the fate and survival of ARB and ARG in effluent water released to the recipient.**

   WWT methods (primary, secondary, tertiary, and quaternary methods) are described in chapter 4.4.3, while the current requirements and state of WWT in Norway are addressed in chapter 4.4.6. Treatment of hospital WW is described in chapter 4.4.5. The fate and survival of ARB and ARG in effluent WW are described in chapters 5.2.5 and 5.3.3, and further discussed in chapter 8. ARB and ARG in hospital WW are specifically described in chapter 5.4.

   There appears to be no significant enrichment of ARB in WWTPs under European conditions. Although some studies indicated a slight increase in the fraction of resistant bacteria, the absolute reduction over treatment is significant, with removals of 99 % to 99.9 % of faecal indicator bacteria generally achieved in Scandinavia with secondary treatment, including biological and physico-chemical treatment steps. Nevertheless, ARB are still released into receiving waters, which may lead to an increased number of faecal indicators, including resistant populations, downstream from WWTP outlets. Therefore, some imprint of AMR in recipient waters, compared to pristine waters, is nevertheless unavoidable.

   There are limited data on the occurrence of ARGs in Norwegian effluent waters. Although markers of resistance towards commonly used antimicrobials in Norway have been found in such effluents, no clear correlation between antimicrobial usage data and the corresponding ARGs have been demonstrated. Some studies have found an increase in relative abundance of ARGs in effluent WW and have speculated that there might be a selective advantage for ARGs (and ARBs) in some WWTP and under certain operating conditions. However, a majority of studies showed a net relative reduction in ARGs suggesting that, overall, WWT tends to reduce the amount of ARGs in effluent.

2. **Describe the sewage sludge treatment methods used in Norway and assess the impact of these methods, on the fate and survival of ARB, ARG, and the content of RD.**

   Sewage sludge treatment methods are described in chapter 4.4.4. The fate and survival of ARB and ARG in sludge are described in chapters 5.2.6 and 5.3.4, and further discussed in chapter 8. Chapter 6 presents data on the occurrence and fate of

antimicrobial agents (i.e., resistance drivers), in effluent WW, hospital WW, sludge, and soil.

There is a large variety of different sludge treatments applied in Norway, ranging from simple long-term storaging to more advanced treatment. It is therefore impossible to say something general about survival and fate of ARB, ARG or RD that occur during these processes. There is a number of single studies reporting diverging results. Many factors may explain this diversity, as endemic levels of AMR and pollutants in the study area, variation in study design and methods applied and focus on different ARB and/or ARG with different characteristics and survival rates. However, it is important to be aware that WWTPs are not generally designed for removal of ARB and/or ARG, but rather for removal of pathogens and/or contaminants.

In general, treatment of sludge significantly reduces the number of faecal indicator bacteria, usually due to increased temperatures or increased pH. For example, thermophilic digestion is superior to mesophilic for such inactivation. Pasteurization of sludge (70 °C for 60 min) will normally kill vegetative bacteria, while spore-forming bacteria survive. Due to the limited amount of studies looking at the ARB fraction in specific species and/or genera that survive or are inactivated, it is impossible to draw any conclusion regarding a potential selection of phenotypic resistance during sludge treatment. Occurrence of AMR has, however, been associated with faecal contamination, and some ARGs have been shown to be higher in treated sewage sludge than in animal manure, food-derived compost, and soil. There are indications that conventional sludge treatment methods are not particularly efficient at ARG removal, although in anaerobic digestion, ARGs were reduced relative to total 16S rRNA gene counts in 21 of 27 observations but increased in 6.

In activated sludge, where the bacterial density is very high, the genes responsible for AMR may spread through bacterial populations via plasmids and a variety of mobile genetic elements. The activated sludge process is the most commonly applied biological wastewater treatment technology, where a bacterial biomass suspension is responsible for the removal of pollutants. However, based on the studies included in this opinion, there is no evidence of such an increase during the biological treatment step of sludge.

Generally, the concentration of ARG in treated sewage sludge seems to be higher than in the untreated sewage. No differences regarding the effects of different sludge treatment methods were shown so far. Up to date there is no evidence at all whether elevated temperatures as under e. g. thermal hydrolysis will be sufficient for ARG inactivation and bring about lower ARG concentrations.

3. **Assess if RDs in fertilising material produced from sewage sludge 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**

Among the resistance drivers in fertilising products produced from sewage sludge, heavy metals like Cu and Zn are already closely surveyed and strictly regulated. Thus, the spread of metals is low and precludes measurable effects on the development, spread and persistence of bacterial resistance against antimicrobial agents (see chapter 8.2).

For organic compounds, concentrations are approx. 2-3 orders of magnitude lower than for metals, and their lifetimes in soil are relatively short (half-lives ranging from days to months). Organic molecules, including antimicrobial agents that are not rapidly degraded, are prone to adsorption and immobilization. This reduces their bioavailability and the risk of leaching to other recipients. Numerous antimicrobial chemicals of natural origin exist in soil, as they are produced by soil microorganisms for gaining competitive advantage or for defence (many antibiotics originate from soil actinobacteria, like *Streptomyces* spp.).

The relative impact of RD on the development of AMR is due to knowledge gaps and uncertainties difficult to evaluate. The most relevant single study comparable to Norwegian conditions is a recent study by Rutgersson et al. 2020, who showed that long-term application of sewage sludge to farmland in Sweden resulted in only minor changes in the composition of the soil bacterial community, and no evidence for enrichment of ARGs or ARBs in soil amended with digested and stored sludge.

4. **Assess the possibility that treated sewage sludge poses a hazard when utilized as a fertilising material in agriculture or in green areas. Also, identify application areas where a hazard for human and animal health or the environment would be expected.**

According to the Norwegian regulations, process control, with specific requirements for temperature and exposure time, is required in the production of sewage sludge. As a verification of treatment and good hygiene practices, fertilising products derived from sewage sludge must also be analysed for bacteriological quality (Lovdata, 2003) (Gjødselvareforskriften – "Norwegian fertilizer regulation"). The process control requirements ensure sufficient bacterial inactivation, including of ARB. Considering the quality demand for sludge hygienisation and stabilisation before use, the maximum use per hectare and year, and the retention time before growth of plants with contact risk, there is very limited likelihood of human and animal exposure to ARB originating from sludge applied as described. Hazards for human, animal, or environmental health due to sludge application are mainly dependent on the

occurrence of resistance drivers in the fertilising product (evaluated in question 3 above), unless heavy rainfall occurs within 18 hours after application and before ploughing (farmers consult weather forecasts and, in practice, abandon spreading sludge under such conditions). The latter could, theoretically, lead to surface water run-off, with the potential for environmental effects.

5. Identify and assess various risk mitigation measures to

- **reduce the probability of wastewater effluent and fertilising material containing ARB?**
- **reduce the probability that wastewater effluent and fertilising material play a role in the development and spreading of AMR.**

Mitigation measures that could be considered are discussed in chapter 10.

All measures that can be taken to avoid dissemination of antibiotics, ARB, and ARG at source should be considered first. Concentrations of antibiotics, ARB, and ARG are highest in the sewage system and at the inlet to the WWTP. Source separation is therefore expected to be very effective. Among these potential measures are education and public awareness campaigns. Furthermore, separation of urine from patients receiving antibiotic treatment (as has been successfully tested for x-ray contrast media), by using pocket urinals, is discussed, and closer evaluation is recommended. Source separation of heavy metals is mentioned here as well.

Due to the high concentrations of antibiotics, ARB, and ARG in the sewage system, risks from leakage are of high concern, although they are currently not quantified. Intrusion of contaminated water into the drinking water distribution system should also raise high concerns. This is especially relevant for Norway because of the "non optimal" condition of the Norwegian sewage and drinking water networks, with leakage rates that are far higher than in other Scandinavian and Central European countries. Rehabilitation of these networks will considerably mitigate risks.

End-of-pipe solutions for the mitigation of the AMR risks include upgrading of WWTP and improving treatment of hospital WW. The level of sewage treatment in Norway is rather low, and upgrading will decrease the concentrations of bacteria, including ARB, further. However, WWTP are generally not designed for removal of AMR, but for the removal of nutrients. Even so-called fourth-treatment stage (quaternary treatment) is designed for removal of trace contaminants. For improving removal of ARB and ARG during WWT, membrane processes seem to be the most promising option.

Finally, it is recommended to question and rethink the concepts of sensitive recipients regarding setting the requirements for the level of WWT. This concept is based on nutrient loads to the environment, rather than on trace contaminants or contaminants such as ARG that develop in a stressed environment. In the future, it might be of

value to define requirements for WWT on the relative contribution of the discharge to the pollution. Using such a paradigm, a small load with contaminants to a rather unpolluted environment would be rated as highly critical and require further treatment, but would not require specific measures under today's regulations.

6. **Identify indicators that can be used for monitoring and control of resistance driving chemicals (antibiotics, antifungal agents, heavy metals, disinfectant agents etc.) in wastewater effluent and sludge destined for use as fertiliser.**

   This issue is addressed in chapter 10. We discuss the possible establishment of a new monitoring programme to run parallel to NORM and NORM-VET: "NORM-ECO". It is emphasized that as NORM and NORM-VET are programmes for monitoring the situation on antimicrobial usage and AMR, and the focus of an additional "NORM-ECO" should be on monitoring as well. There is still relatively little knowledge on RD and AMR in non-clinical compartments, and parameters that would trigger an immediate response from NFAS or NEA have not yet been identified.

7. **How significant is the exposure of workers, farmers and the public to AMR through production and use of sludge as a fertiliser material in Norway?**

   No Norwegian data or information about this issue have been found. Although, such risks are regarded as low, they cannot be discounted.

8. **Evaluate the prevalence of ARB and ARG in wastewater effluent in different WWTPs with low and high exposure of potential resistance drivers (hospitals, industry, universities and household).**

   Scientific studies of effluent WW in European WWTPs have verified that the levels of ARG and ARB are reduced by sewage treatment in the WWTP. Countries with the highest consumption of antibiotics also have the highest levels of ARG in effluent water. Some resistance genes, such as vancomycin-resistance genes and sulphonamide-resistance genes, seem to be enriched to some degree in all WWTP, independent of the level of use of antibiotics in the society. There is a link between the occurrence of groups of ARG in the effluent water due to genetic linkage and this can be used to simplify monitoring the levels of ARG in the effluent water of WWTPs. Recent scientific results link the occurrence of certain bacterial species to certain ARG in effluent water. This can indicate that both antibiotic usage and antibiotic residues can result in certain bacterial groups attracting and spreading ARGs, and can then be selected further in the WW treatment infrastructure. Hospital WW has more ARB, ARG, and antibiotic residues than community 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 create a higher impact on the effluent water from the WWTP. In such cases, local treatment of the WW onsite at the hospital could be beneficial.

9. Describe the biological characteristics of the ARB and ARG identified in WWTPs

This is described in chapter 6 and further discussed in chapter 8.

13 Data gaps

In this chapter, insufficient knowledge and/or data related to the topic covered in the assessment is described. All data gaps described were uncovered during the assessment process.

Table 13-1. Data gaps identified in this assessment

Data gaps	Expected impact in the event of filled data gaps (for VKM, the assigner, and/or the society)
Research is needed regarding occurrence of PTM resistance in bacteria in wastewater	Data collection and development of standard methods will enable the proper characterization of the potential hazard for development of resistance/reduced susceptibility in bacteria. This would enable risk managers to make informed decisions in future measures and regulations.
Research is needed regarding occurrence of disinfectant resistance in bacteria in wastewater	As above
Research is needed regarding cocktail effect of antimicrobial agents (antibacterial, antifungal, PTM, disinfectant agents) for development of resistance in bacteria in wastewater	Research on potential synergistic and antagonistic effects of mixtures of "cocktail effect" of antimicrobial agents in developing of resistance and what kind of resistance in bacteria, in wastewater. This data will be highly beneficial to risk assessors for evaluation of potential risks (hazards).
Research is needed in order to identify and quantify the sources, occurrence and transport of antimicrobial agents residues (antibacterial agents, antifungal agents, disinfectant agents, potential toxic metals, and other substances), ARB, and ARG to environmental media like water, wastewater and other media to which human/animals are exposed.	Identification and quantification of the sources would help risk assessors to identify "hot spots" and recommend specific mitigations to risk managers.
Research is needed regarding wastewater treatment technologies; physical, chemical, biological treatments to minimize antimicrobial agents' residues and their metabolites, ARB, and ARG	New and better technologies would help risk manager to reduce AMR in the environment.

Further studies are needed to determine the precise values of the abundance of antimicrobial agents' residues and their metabolites, ARB, and ARG in WWTP discharges that do not trigger human health issues (thresholds).	Such information is important for risk assessors to evaluate potential risks (hazards) and propose mitigations to risk managers.
Further studies are needed to determine the fate of the most extensive spectrum of antimicrobial agents like fluoroquinolones and tetracyclines resistance in the environment	This information is essential for antimicrobial agents like fluoroquinolones and tetracyclines, which are the most stable antibacterial agents in the environments.
There is a need for the development and implementation of an "indicator" system to identify and quantify sentinel AMR bacteria in water, wastewater and other exposure media, regularly and widely. **Establishment of a "ECO-NORM"-system requires clarification of many questions that only can be answered by further research.**	Implementation of an "indicator" system, like NORM/NORM-VET would contribute to monitor AMR bacteria in the environment. An equivalent system like NORM/NORM-VET. Availability of such monitoring system is important for use in the attainment of holistic risk assessments.
Exposure of workers	There is lack of knowledge concerning the exposure of workers and farmers to AMR through production and use of sludge as a fertilising product in Norway.

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