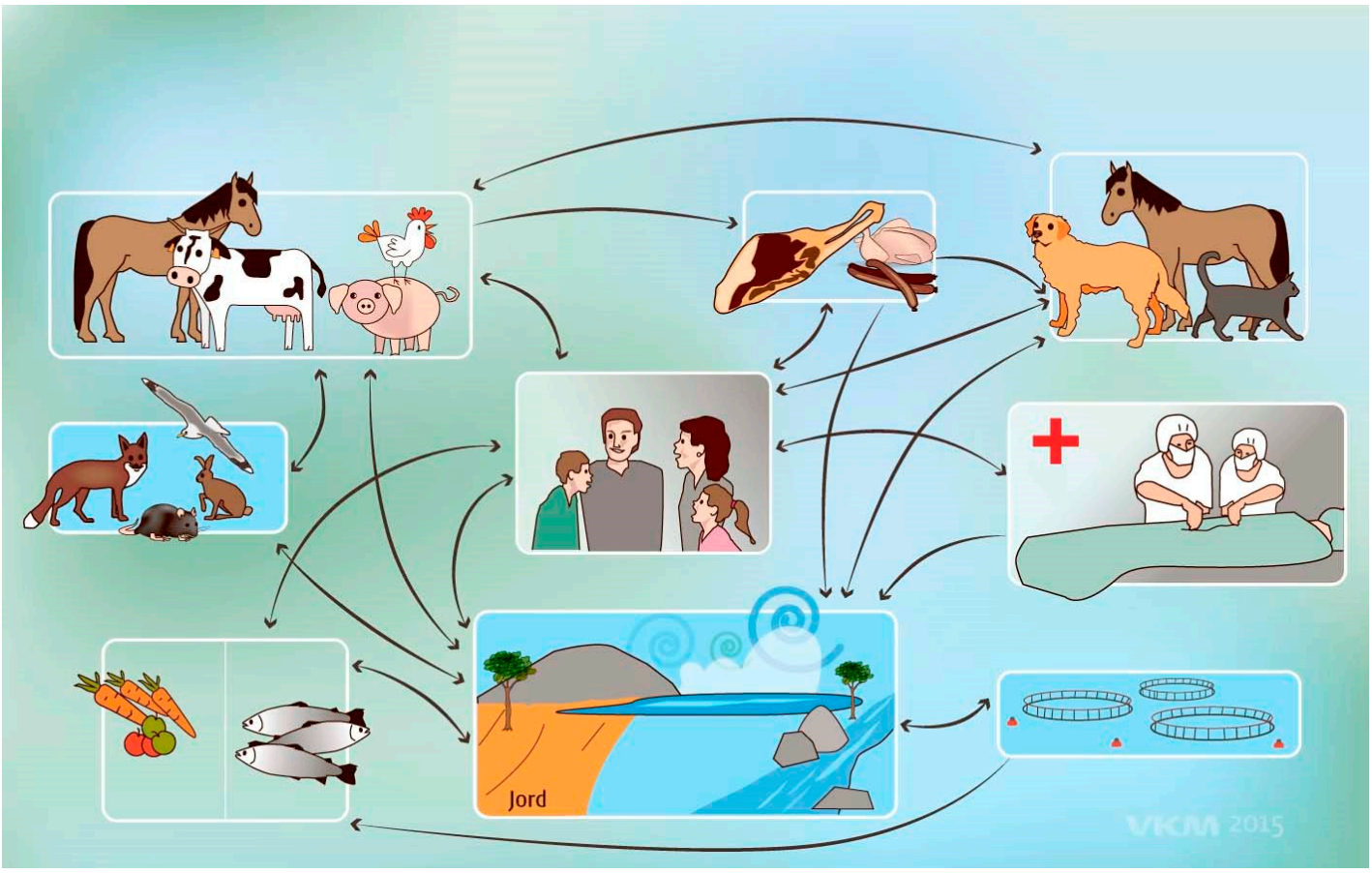
Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety



VKM Report 2015:29

Assessment of antimicrobial resistance in the food chains in Norway

Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2015: 29
Assessment of antimicrobial resistance in the food chains in Norway

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Assessment of antimicrobial resistance in the food chains in Norway

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Assessed and approved

The opinion has been assessed and approved by the Panel on Biological Hazards of VKM. Members of the panel are: Yngvild Wasteson (chair), Karl Eckner, Georg Kapperud, Jørgen Lassen, Judith Narvhus, Truls Nesbakken, Lucy Robertson, Jan Thomas Rosnes, Taran Skjerdal, Eystein Skjerve, Line Vold, Siamak Yazdankhah.

Acknowledgments

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority/Norwegian Environment Agency. The project leader from the VKM secretariat has been Danica Grahek-Ogden. The members of the working group, Siamak Yazdankhah, Brit Hjeltnes (National Veterinary Institute; VI), Solveig Langsrud (NOFIMA), Jørgen Lassen, Madelaine Norström (VI), Marianne Sunde (National Public Health; FHI), and Yngvild Wasteson, are acknowledged for their valuable work on this opinion. VKM acknowledges the Panel on Animal Health and Welfare for commenting on the draft of this risk assessment. Roar Gudding is acknowledged for his valuable comments and views. VKM would like to thank the hearing experts Gunnar Skov Simonsen (University Hospital of North Norway; UNN).

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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Summary

The Norwegian Food Safety Authority (NFSA) asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) for an assessment of antimicrobial resistance (AMR) in the food chains in Norway, with focus on each of the following food chains: pigs and pork products; poultry, eggs and poultry products; cattle and bovine products; aquaculture and aquaculture products; fresh produce (fruit, berries, and vegetables); and drinking water.

AMR in imported food has not been assessed in this report. AMR in Norwegian food chains has been assessed in terms of probability of exposure to humans. Due to data constraints, it has not been possible to assess the consequences of this exposure for human health.

VKM appointed a working group consisting of three members of the Panel on Biological Hazards, one member of Panel on Animal Health and Welfare, and four external experts to prepare a draft Opinion document and the answer the questions. The Panel on Biological Hazards has reviewed and revised the draft prepared by the working group and approved the Opinion document «Assessment of antimicrobial resistance in the food chains in Norway".

AMR can be described as the ability of a bacterium to withstand the effects of an antimicrobial. The clinical antimicrobial resistance crisis has focused attention on all uses of antimicrobial agents, including their use in human medicine, veterinary medicine, and in agriculture and aquaculture. AMR is considered the greatest challenge to face health care in 21st century, and there is increasing concern and debate about which roles the food production chains play as reservoirs and disseminators of AMR.

This assessment addresses several food chains. The report does not characterise all forms of AMR that may occur in these chains, but puts emphasis on the resistant bacteria and resistance determinants that have emerged at the animal-human interface in recent decades. VKM's choice is based on zoonotic potential and the limited alternatives available for treatment of infections. In order for a comprehensive and detailed assessment to be conducted, these particular resistance forms need to be characterised and assessed separately.

At an overall level, the hazard regarding exposure of humans to antimicrobial resistant bacteria from cattle, milk/milk products, fish/fish products/seafood, fresh produce, water, and food processing in Norway is considered by VKM to be negligible.

Current data regarding possible pathways for transmission of LA-MRSA via contaminated food/meat to the broader human population fail to implicate LA-MRSA from pigs as a

foodborne pathogen. Compared with other animal products, poultry and poultry products are regarded as the most important reservoirs of ESBL/AmpC-producing *Enterobacteriaceae*, quinolone-resistant *E. coli* (QREC), and their corresponding resistance determinants. The probability of human exposure of ESBL/AmpC-producing *Enterobacteriaceae* and QREC via poultry is assessed as being non-negligible.

Probability of AMR transfer associated with food and uncertainties

In this assessment, the probability of transmission of AMR from food chains to humans has been either categorized as negligible or non-negligible according to the following definitions:

- Negligible – the probability of transfer of AMR is extremely low. Negligible probability should be considered insignificant.
- Non-negligible – the probability of transfer of AMR is greater than negligible. Non-negligible probability should be considered significant, but the available data are currently insufficient to enable discrimination between the different levels.

Lack of data has made it difficult to reach any firm conclusions regarding the probability of AMR transmission from food to humans in Norway. Similarly, ranking the probabilities with regard to relative importance is largely not possible with the data available.

The probability of transfer of AMR from cattle, milk/milk products, fish, seafood, and drinking water has been assessed to be **negligible**.

The probability of transfer of LA-MRSA from live pigs to humans is considered to be **non-negligible**, while the probability of transfer from pork to humans has been assessed to be **negligible**.

The probability of transfer of ESBL/AmpC-producing *Enterobacteriaceae*, quinolone-resistant *E. coli,* and their respective corresponding genes from live poultry and poultry meat is considered as **non-negligible**.

Processing of food, such as cooking or preservation, can reduce the number of bacteria in the products and thus decrease the transmission of antimicrobial resistant bacteria from food to humans.

It should be noted that both categories of probabilities (negligible and non-negligible) in this assessment are associated with a number of uncertainties. Bacteria are living organisms that are under continuous evolution, and are able to adapt rapidly to changing living conditions. This report is an assessment of the current situation with regards to development and dissemination of antibiotic resistant bacteria and their resistance genes in the food chain. This situation may change as the bacteria continue to adapt to the selection pressures exerted by the worldwide use of antimicrobials. Such bacterial changes, sometimes occurring

in “quantum leaps” due to horizontal gene transfer (HGT), may also rapidly change the probability of transfer of resistance to specific antimicrobials.

Data gaps

There is a lack of knowledge regarding the vast reservoir of AMR in the environmental, animal, and food reservoirs. Furthermore, there is lack of data regarding the routes and frequencies of transmission of AMR from live, food-producing animals and foodstuffs of different origins to humans and vice versa.

Key words: VKM, assessment, Norwegian Scientific Committee for Food Safety, biological hazards, antimicrobials, resistance, MRSA, VRE, QR, ESBL/AmpC

Sammendrag på norsk

Mattilsynet ba Vitenskapskomiteen for mattrygghet (VKM) om en vurdering av antimikrobiell resistens i matkjeden i Norge. Spørsmål fra Mattilsynet besvares for hver av de følgende næringskjeder: gris og svinekjøttprodukter, fjørfe, egg og fjørfeprodukter, storfe og storfeprodukter, akvakultur og akvakulturprodukter, frukt, bær og grønnsaker, og vann.

Antimikrobiell resistens i importert mat er ikke vurdert i denne rapporten, siden det ikke ble spurt om av Mattilsynet. Risiko for antimikrobiell resistens i norske matkjedene er vurdert som sannsynligheten for at mennesker blir eksponert for de ulike resistensformene. På grunn av utilstrekkelig datagrunnlag har det ikke vært mulig å vurdere konsekvensene av denne eksponeringen.

VKM nedsatte en prosjektgruppe bestående av medlemmer av Faggruppen for hygiene og smittestoffer, ett medlem av Faggruppen for dyrehelse og velferd og fire eksterne eksperter til å forberede svar på spørsmål. Faggruppen for hygiene og smittestoffer har gjennomgått og revidert utkast utarbeidet av prosjektgruppen og endelig godkjent vurdering.

Antimikrobiell resistens kan beskrives som bakterienes evne til å motstå virkningen av antimikrobielle stoffer, og regnes som en av de største utfordringene i helsevesenet i det 21. århundre. En svært viktig årsak til utvikling og spredning av resistens er bruk av antimikrobielle midler. Det er derfor vesentlig å se på all bruk av antimikrobielle stoffer, inkludert bruk i humanmedisin, veterinærmedisin og i jordbruk og akvakultur, når problemstillinger knyttet til resistens skal diskuteres.

Et økende fokus på hvilken rolle matproduksjon spiller som et reservoar av antimikrobiell resistens danner et bakteppe for denne rapporten. VKM har som svar på Mattilsynets oppdrag gjort en overordnet vurdering av flere sammensatte matkjeder. Det har i dette arbeidet ikke vært mulig å vurdere alle mulige resistensformer som kan finnes i matproduserende dyr og i mat. VKM har fokusert på de resistente bakteriene og resistensdeterminantene som er mest aktuelle med tanke på zoonotisk potensiale, samt de resistensformene som det finnes få alternative antibiotika overfor. Det er imidlertid nødvendig å adressere hver av disse resistensformene separat dersom det skal gjøres en mer detaljert og omfattende risikovurdering.

I denne vurderingen er sannsynligheten for overføring av antimikrobiell resistens fra matkjeden til mennesker kategorisert enten som neglisjerbar (ekstremt lav) eller ikke-neglisjerbar (større enn neglisjerbar). Det må tas i betraktning at begge kategorier av sannsynligheter (neglisjerbar og ikke neglisjerbar) er forbundet med en rekke usikkerhetsmomenter.

På et overordnet nivå har VKM vurdert sannsynligheten er neglisjerbar for at mennesker eksponeres for antimikrobiell resistens fra storfe- og storfekjøtt, melk og melkeprodukter, fisk, fiskeprodukter og sjømat, grønnsaker, frukt og bær, samt drikkevann som er produsert i Norge.

Ut ifra tilgjengelige norske data vurderes det som sannsynlig at mennesker kan eksponeres for LA-MRSA fra griser som er bærere av denne type bakterier, det vil si at sannsynligheten kategoriseres som ikke-neglisjerbar. Sannsynligheten for at mennesker blir eksponert overfor LA-MRSA via svinekjøtt regnes imidlertid som neglisjerbar.

Fjørfe og fjørfeprodukter regnes som det viktigste reservoaret for ESBL/AmpC-produserende *Enterobacteriaceae*, kinolonresistente *E. coli* (QREC) og deres respektive resistensdeterminanter sammenlignet med andre animalske produkter. Sannsynligheten for at mennesker eksponeres overfor ESBL/ AmpC-produserende *Enterobacteriaceae* og QREC via fjørfe og fjørfeprodukter vurderes som ikke-neglisjerbar.

Kommersiell foredling av mat og matlaging kan redusere sannsynligheten for overføring av resistente bakterier fra matkjeden til menneske. Sannsynligheten reduseres fordi mange former for bearbeiding av mat, slik som f. eks. ulike konserveringsmetoder og varmebehandling reduserer det totale antall bakterier i maten. Dette forutsetter imidlertid at brudd på hygienerutiner ikke forårsaker ny forurensing av maten underveis i produksjonen eller hjemme på kjøkkenet.

Datamangler

Det er stor mangel på kunnskap om det omfattende reservoaret av antimikrobiell resistens som finnes hos mennesker og dyr og i miljøet. Videre er det mangel på forståelse for effekten av de viktigste drivkreftene for utvikling og spredning av resistens, samt data om de mest effektive veiene for overføring av antimikrobiell resistens fra levende dyr og mat av forskjellig opprinnelse til mennesker og vice versa.

Abbreviations and glossary

Abbreviations

aEPEC	Atypical enteropathogenic *E. coli*
AGP	Antimicrobial growth promotion/promoters
AMR	Antimicrobial resistance
ARB	Antimicrobial resistant bacteria
ARG	Antimicrobial resistant gene
BIOHAZ	EFSA's Panel on Biological Hazards
BSI	Bloodstream infections
CAC	Codex Alimentarius Commission
CA-MRSA	Community-acquired methicillin-resistant *Staphylococcus aureus*
CC	Clonal complex
CIA	Critically important antimicrobial agent
CoNS	Coagulase-negative staphylococci
CP	Carbapenemase-producing
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological cut-off value
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESBL	Extended-spectrum beta-lactamase
EUCAST	European Committee for Antimicrobial Susceptibility Testing

FAO	Food and Agricultural Organisation of the United Nations
HA-MRSA	Hospital-acquired methicillin-resistant *Staphylococcus aureus*
HGT	Horizontal gene transfer
HTST	High-temperature, short-time
LAB	Lactic acid bacteria
LA-MRSA	Livestock-associated methicillin-resistant *Staphylococcus aureus*
MATS	Norwegian Food Safety Authority's form service
MDR	Multidrug resistant
MIC	Minimum inhibitory concentration
MLST	Multi-locus sequence typing
MRL	Maximum residue limits
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin susceptible *Staphylococcus aureus*
NFSA	Norwegian Food Safety Authority
NORM	The Norwegian monitoring programme for AMR in human pathogens
NORM-VET	The Norwegian monitoring programme on AMR in bacteria from food, feed, and animals
NWGA	Norwegian Reference Group on Antibiotic Susceptibility Testing
OIE	The World Organization for Animal Health
PMQR	Plasmid-mediated quinolone resistance
QACs	Quaternary ammonium compounds
QPS	Qualified presumption of safety
QRDR	Quinolone-resistance determining region
QREC	Quinolone-resistant *E. coli*

SCENIHR	The Scientific Committee on Emerging and Newly Identified Health Risks
SSI	Statens Serum Institute
ST	Sequence type
STEC	Shigatoxin-producing *E. coli*
STP	Sewage treatment plant
UHT	Ultra high temperature
UNICEF	United Nations Children's Fund
UPEC	Uropathogenic *E. coli*
UTI	Urinary tract infections
VESUV	Norwegian outbreak surveillance programme (**ve**vbasert **s**ystem for **u**tbrudds**v**arsling)
VetReg	Register of prescriptions issued by veterinarians
VKM	Norwegian Scientific Committee for Food Safety
VMP	Veterinary medicinal product
VRE	Vancomycin-resistant Enterococci
WHO	World Health Organization

Glossary

Acquired resistance: Resistance to a particular antimicrobial agent to which the microorganism was previously susceptible. The change 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 and that act in low concentrations against other bacterial species. Today “antibiotics” comprises also synthetic and semisynthetic compounds 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 is defined as (Davison et al., 2000):

A property of bacteria that confers the capacity to inactivate or exclude antimicrobials, or a mechanism that blocks the inhibitory or killing effects of antimicrobials.

The ability of a microorganism to withstand an antimicrobial.

A relative term that provides an interpretation of the clinical significance of concentrations of an antimicrobial that inhibits the growth of an organism or kills it in laboratory systems (*in vitro*).

Either microbiological resistance, where resistant organisms are those that possess any kind of resistance mechanism or resistance gene, or clinical resistance, where a bacterium is classified as susceptible or resistant depending on whether an infection with that bacterium responds to therapy or not.

Bactericidal agent: An antimicrobial agent capable of killing bacteria.

Biocide / Biocidal products: Active substances and preparations containing one or more substances, in the form in which they are supplied to the user, 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. Flocs are suspended aggregates of microorganisms surrounded by an extracellular polymeric slime matrix that is formed in liquid suspension.

Clinical breakpoints: NORM and NORM-VET data are categorized according to the breakpoints of the Norwegian Reference Group on Antibiotic Susceptibility Testing (NWGA). These breakpoints are harmonized with EUCAST breakpoints that define the terms clinically susceptible and clinically resistant (http://www.srga.org/Eucastwt/eucastdefinitions.htm).

Clone: Bacterial isolates that, although they may have been cultured independently from different sources in different locations and perhaps at different times, still have so many identical phenotypic and genotypic traits that the most likely explanation for this similarity is a common origin within a relevant time span.

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 located together on a mobile genetic element (such as a plasmid, transposon, or integron).

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

Disinfection: Use of physical procedures or chemical agents (disinfectants) to destroy most microbial forms (mainly on inanimate material, but also on skin surfaces). Disinfectants are often not effective against bacterial spores.

Epidemiological cut-off values (ECOFF): The purpose of the ECOFF values is to distinguish between the wild type populations and non-wild type populations, the latter of which is defined as microorganisms with acquired resistance mechanisms to an agent (further information: http://www.srga.org/Eucastwt/eucastdefinitions.htm)

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

Intrinsic resistance: A natural property of an organism resulting in decreased susceptibility to a particular antimicrobial agent.

Isolate: A bacterial isolate can be defined as a single isolation in pure culture from a specimen.

Microbiota: Collective term for microflora (i.e., any type of minute organism) 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. MIC data can provide information about the activity of antimicrobials.

Multi Locus Sequencing Typing (MLST including ST): is a procedure for characterizing isolates of bacterial species using the sequences of seven housekeeping genes. Approximately 450-500 base pair internal fragments of each gene are used, as these can be accurately sequenced. For each housekeeping gene, the different sequences present within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of the seven loci define the allelic profile or sequence type (ST).

Normal flora: Indigenous microbial flora of human/animal external and internal surfaces like the skin, mouth, and gastrointestinal tract, and the upper respiratory tract. The normal flora contains numerous bacterial species, and numerous strains within each species. Although it may contain opportunistic pathogens, the vast majority are symbiotic or commensals that contribute to general health as well as to colonization resistance. However, some of these low-virulence bacteria of the normal flora may, under certain circumstances, become opportunistic pathogens.

Sanitizer: An agent that reduces microbiological contamination.

***spa*-typing**: Typing *Staphylococcus aureus* by using the short sequence repeat region of the protein A (spa) gene has been suggested to work as well as the MLST method. *spa* typing has significant advantages in terms of speed, ease of use, standardization, and reproducibility as compared with the MLST method and other techniques.

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

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

Strain: A subset of a bacterial species differing from other bacteria of the same species by some minor, but identifiable, difference.

Susceptibility: Describes the extent to which a target microorganism is affected by an antimicrobial agent.

Transduction: Transfer of genetic material from one bacterium to another via bacteriophages (viruses that infect bacteria and are integrated into the host genome).

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

Background as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) is developing a strategy on preventing or reducing the further development and spread of antimicrobial resistance (AMR). In order to provide the basis for the strategy, the Norwegian Scientific Committee for Food Safety (VKM) is asked to address the impact on human health from the spread of resistant bacteria and/or antimicrobial resistance. The assessment should include routes through animals and food chains, directly or indirectly, at present and in the future. This should include, although from a broad perspective, the significance of imported live food-production animals, produce and commodities, ingredients, and products. It should also assess the significance of these routes in the total load of antimicrobial resistant bacteria to which humans are exposed in Norway. Where knowledge critical for the development of a future-oriented strategy in this field is insufficient, the Committee is asked to describe these knowledge gaps.

Terms of reference as provided by the Norwegian Food Safety Authority

From this perspective the NFSA asks VKM to answer the following questions:

1. Which antimicrobial resistant bacteria/resistance genes are/will be of most importance regarding transfer from the food chains (directly from any part of the food chains and from the food itself) to humans in Norway?
2. Where and how are resistant bacteria and/or AMR introduced to, or induced in, the food chains, and how are they transferred through the food chains to humans?
3. To what extent will exposure through the food chains contribute to the total load of each of the most important resistance forms in humans?

These questions should be addressed for each of the following food chains:

a) Pig production and products
b) Poultry and egg production and products
c) Cattle production and products
d) Aquaculture and aquaculture products
e) Fresh produce (fruit, berries, and vegetables)
f) Water

1 Literature

1.1 Relevant background papers provided by the Norwegian Food Safety Authority

Audit data from the NFSA's supervision of animal health personnel (MATS/VetReg)

NFSA's data and experiences from the work on the eradication of MRSA (June 2014) (In Norwegian).

Report on antibiotic resistance - Challenges and proposals for action by sectoral experts (June 2014) (http://www.fhi.no/dokumenter/35ed0e4c20.pdf).

Data from contaminants programme
http://www.vetinst.no/Publikasjoner/Rapportserie/Rapportserie-2014/Fremmedstoffprogrammet-2013

1.2 Literature searches performed in PubMed

General information regarding the modes of action of antimicrobial agents, AMR, and horizontal gene transfer (HGT) was obtained by using search: antimicrobial resistance [Title/Abstract]) OR antibiotic resistance[Title/Abstract]) AND Review[ptyp])). Only the articles published in the last 10 years were used in this assessment.

Literature on AMR in zoonotic agents was obtained using search: salmonella[Title/Abstract]) OR campylobacter[Title/Abstract]) OR listeria[Title/Abstract]) AND resistance[Title/Abstract])) AND food[Title/Abstract] Filters: Review

Literature on AMR in food-producing animals (pigs, poultry, cattle) was provided using searches:

Food-producing animals (pigs, poultry, cattle, fish)

(food-producing[All Fields] AND ("animals"[MeSH Terms:noexp] OR animals[All Fields])) AND (("anti-infective agents"[All Fields] OR "anti-infective agents"[MeSH Terms] OR ("anti-infective"[All Fields] AND "agents"[All Fields]) OR "anti-infective agents"[All Fields] OR "antimicrobial"[All Fields]) AND resistance[All Fields])

(("anti-infective agents"[All Fields] OR "anti-infective agents"[MeSH Terms] OR ("anti-infective"[All Fields] AND "agents"[All Fields]) OR "anti-infective agents"[All Fields] OR

"antimicrobial"[All Fields]) AND resistance[All Fields]) AND ("cattle"[MeSH Terms] OR "cattle"[All Fields])

("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) AND ("cattle"[MeSH Terms] OR "cattle"[All Fields])

("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) AND pig

("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) AND swine

("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) AND ("poultry"[MeSH Terms] OR "poultry"[All Fields]) AND poultry

(("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) AND (("food"[MeSH Terms] OR "food"[All Fields]) AND producing[All Fields])) AND ("norway"[MeSH Terms] AND "norway"[All Fields])

("egg shell"[MeSH Terms] OR ("egg"[All Fields] AND "shell"[All Fields]) OR "egg shell"[All Fields]) AND (("anti-infective agents"[Pharmacological Action] OR "anti-infective agents"[MeSH Terms] OR ("anti-infective"[All Fields] AND "agents"[All Fields]) OR "anti-infective agents"[All Fields] OR "antimicrobial"[All Fields]) AND resistance[All Fields])

milk [Title/Abstract]) AND antibiotic resistance [Title/Abstract] AND Norway [Title/Abstract]

cheese [Title/Abstract]) AND antibiotic resistance [Title/Abstract]

Literature regarding fish/fish products and AMR was provided using search: Fish[Title]) OR seafood[Title]) AND antimicrobial resistance[Title]) OR antibiotic resistance[Title] Filters: Review, 10 years, Other Animals

Literature regarding vegetables/fruit was provided using search: fruit*[Title]) OR vegetable*[Title]) AND resistance[Title/Abstract] Filters: 10 years

Literature regarding water was provided using search: water OR drinking water AND antibiotic resistance (Title) Filters: Review

Literature regarding food processing/disinfectant agents was provided using search: antimicrobial resistance[Title/Abstract]) OR antibiotic resistance[Title/Abstract]) AND food processing[Title/Abstract]

((((((antimicrobial resistance[Title/Abstract]) OR antibiotic resistance[Title/Abstract]) AND food[Title/Abstract]) OR food chain[Title/Abstract]) AND Review[ptyp])) AND microorganisms[Title/Abstract]

1.3 Other sources of information

Antimicrobial agents used in food-producing animals in Norway: Information regarding antibacterial agents used in food-producing animal with marketing authorization was provided from the database at the Norwegian Medicines Agency.

1.4 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. The reference lists in selected citations were scrutinized to identify additional articles or reports that had not been identified by the PubMed searches.

2 Introduction

2.1 Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is a major threat to global health. In clinical medicine, the development of AMR in human pathogens has been widely publicized and is recognized as a major threat to the control of bacterial infections worldwide and modern medicine in general (Levy, 1992; WHO, 2014). In Europe, the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) have estimated that more than 25 000 extra deaths annually are associated with AMR. The economic burden of this amounts to € 1.534.100.000 annually (ECDC/EMEA, 2009). AMR has been described for all known antimicrobials currently available for clinical use and this development may result in a major public health crisis due to the return of untreatable infections on a massive scale.

The clinical AMR crisis has focused attention on all uses of antimicrobials, including their use in human medicine, veterinary medicine, and in agriculture and aquaculture. There is increasing concern and debate about which roles the food production chains play as reservoirs and disseminators of AMR (CIWF, 2011; Merle et al., 2012).

Restricting both therapeutic and prophylactic uses of antimicrobials in clinical settings and food production has been the primary strategy for AMR mitigation (Wang et al., 2012). However, despite these efforts, the trend of rising AMR continues. Antimicrobial use is a double-edged sword and AMR is a complicated issue. Effective mitigation will require targeted strategies built upon a comprehensive understanding of AMR emergence, amplification, dissemination, persistence, and circulation (Wang et al., 2012).

Several studies of ancient bacterial DNA conclude that AMR is a natural phenomenon among environmental bacteria that pre-dates the selective pressure of the massive use of antimicrobials in our time (D'Costa et al., 2011). During their evolution, bacteria developed various AMR mechanisms in parallel to the biosynthesis of antibacterial substances produced by other organisms in their environment or by themselves (Finley et al., 2013). These environmental bacteria are regarded as being the major source of AMR in clinically relevant bacteria, and the massive use of antimicrobials has selected a subset of these resistance genes that now appear to be widely distributed in nature and that challenge modern medicine (Martinez, 2014).

The emergence of AMR is a core issue for the One Health Initiative, which was launched as "the collaborative effort of multiple disciplines — working locally, nationally, and globally — to attain optimal health for people, animals and the environment" (AVMA, 2008). The concept has been adopted by FAO, OIE, WHO, the UN System Influenza Coordination,

UNICEF, and the World Bank as a strategic framework for reducing risks from infectious diseases at the animal-human-ecosystems interface (http://www.fao.org/docrep/011/aj137e/aj137e00.HTM).

2.2 Classification of antimicrobials according to their importance in human medicine

In 2005, the WHO organized a consultation in Australia to develop a list of antimicrobial agents in human medicine. This list divided the antimicrobial agents used in human medicine into three different categories:

- Critically important antimicrobials,
- Highly important antimicrobials, and
- Important antimicrobials

Each antimicrobial agent (or class) was assigned to one of three categories of importance on the basis of two criteria:

a. the agent or class is the sole therapy or one of few alternatives to treat serious human disease; and
b. the antimicrobial agent or class is used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources.

The 3 categories were:

Critically important antimicrobials are those that meet both criteria.

Highly important antimicrobials are those that meet 1 of the 2 criteria.

Important antimicrobials are those that do not meet either criterion.

This list was generated in an effort to provide a tool for developing risk-management strategies and focusing resources to address antimicrobial use in agriculture and veterinary medicine. Until that time, there had been no international consensus on the classification according to importance of different groups of antimicrobial agents. The WHO convened a second meeting in Copenhagen, Denmark, in 2007 to re-evaluate the classification of antimicrobials and update the list on the basis of recent developments. Relatively few changes were needed. Table 12-1 shows the different categories of antimicrobial agents used in human medicine (WHO, 2012).

Similarly, OIE has ranked veterinary antimicrobial agents as critically important, highly important, or important to animal health, according to the same criteria as the WHO. When the lists of critically important antimicrobials are examined, some classes appear only on the WHO list (carbapenems, ansamycins, glycopeptides, streptogramins, and oxazolidinones), whereas other classes appear only on the OIE list (phenicols, sulphonamides, diaminopyrimidines and tetracyclines). However, for a number of classes there is an overlap, such that the class of antimicrobial agents is listed as critically important for human health by WHO and critically important for animal health by OIE. These are 3rd and 4th generation cephalosporins, quinolones (including fluoroquinolones), macrolides, penicillins, and aminoglycosides. This overlap highlights the need for AMR surveillance, and to be able to identify and implement appropriate management measures in order to mitigate resistance dissemination and maintain the efficacy of the drugs. Prudent use of all antimicrobials is considered essential (FAO/WHO, 2008).

2.3 Use of antimicrobial agents in food-producing animals, fish, and plants

2.3.1 Globally

FAO, OIE, and WHO have organized a number of consultations to address the issues related to antimicrobial use in food-producing animals, fish, and plants, the emergence of resistant pathogens in food chains, and the potential public health impacts (FAO/OIE/WHO 2006).

Antimicrobials are administered to animals for a variety of reasons: disease treatment, disease prevention and disease control, and growth promotion/feed efficiency. They are predominantly used to treat respiratory and enteric infections in groups of intensively fed animals, especially during the early part of an animal's life – for example, for flock treatment of broilers, weaning pigs, and calves (Phillips et al., 2004). Antimicrobials are also used to treat infections in individual animals caused by a variety of bacterial pathogens, in particular to treat mastitis in dairy cows. The global increase in fish farming and aquaculture was accompanied by bacterial infections that were usually treated with antimicrobial agents added to fish feed. Today, these diseases are largely controlled by vaccines.

Bacterial diseases, although less prevalent than diseases caused by fungi or viruses, can cause severe constraints to crop production. Antimicrobials have therefore been regarded as essential in many countries for control of certain bacterial diseases of high-value fruit, vegetables, and ornamental plants (McManus et al., 2002; WHO, 2011). Countries where antimicrobials are registered for use in plant agriculture include: USA, Israel, New Zealand, Canada, Mexico, and – strictly regulated on an emergency-use permit basis only - also in Germany, Austria, and Switzerland (Stockwell and Duffy, 2012). The antimicrobials mostly used are streptomycin and oxytetracycline, primarily for control of fire blight on pears and

apples caused by *Erwinia amylovora*, and also against “bacterial spot” of stone fruits (e.g. peaches and nectarines) caused by *Xanthomonas arboricola*.

Antimicrobial growth promotion (AGP) was first advocated in the 1950s, when it was discovered that small sub-therapeutic quantities of antimicrobials, such as procaine penicillin and tetracycline (1/10 and 1/100 the amount of the therapeutic doses), administered to animals in feed, could enhance the feed : weight ratio for poultry, pigs, and beef cattle (Stokestad and Jukes, 1950). Use of antimicrobials for AGP has been banned in the EU since 1998, but is still in use in other countries such as USA.

2.3.2 In Norway

Only authorized veterinarians can prescribe veterinary medicinal products (VMP) for treatment of animals (Masters in Aquamedicine can also prescribe VMP for fish) (LOVDATA, 2001b). Only therapeutic agents that have been evaluated and approved in accordance with EU regulations can be administered (LOVDATA, 2007). For each substance and animal group, Maximum Residue Limits (MRLs) have been established (LOVDATA, 2012).

The usage of VMP for therapeutic use in food-producing animals in Norway is low compared with other countries (Table 12-2, Figure 2-1 and Figure 2-2) (EFSA/ECDC, 2013; NORM/NORM-VET, 2013). In 2014, the total sales of antimicrobial VMP for terrestrial animals were 5,927 kg, which included use in both food-producing animals and companion animals. Use of antimicrobial agents and development of resistance in bacteria from companion animals (excluding horses) has been discussed in another Opinion assessment (VKM, 2015a). The annual sales, in kilogram active substance, of antimicrobial VMP approved for use in terrestrial animals decreased by approximately 38 % from 1995 to 2014. Information regarding the amount of antimicrobial agents used for therapeutic purposes in different species of food-producing animals in Norway is not available.

The sales of antimicrobial VMP in Norwegian aquaculture declined by approximately 99 % from 1987 to 1996 and have, thereafter, remained relatively constant. In 2014, the total sales of antimicrobial agents for therapeutic use in farmed fish were 511 kg of active substance, of which amphenicols accounted for 79 %.

The use of AGP in Norway ceased in 1995 when the livestock industry instituted a self-imposed ban on their use. The veterinary authority in Norway adopted the ban on the use of avoparcin as a growth promoter in animals from 1. June 1995.

In 2014, the total sales of ionophore coccidiostat feed additives were 13 722 kg of active substance, more than four times the amounts used prior to the withdrawal of AGP in 1995. This is explained by increased production of broilers. Monensin was the most frequently used ionophore in poultry in 1995.

The usage of coccidiostats has since been dominated by narasin (12 409 kg in 2014), whereas monensin (1 313 kg in 2014) is used in turkey production (NORM/NORM-VET, 2014). Narasin (originally patented as an antibiotic) has both antibacterial and coccidiostatic activity (Kastner and Hamill, 1982). Coccidiostat agents have been evaluated in a separate assessment (VKM, 2015b). During 2015, the poultry industry started to produce broilers without use of narasin on a larger scale.

Table 12-2 shows the different antimicrobial agents used in food-producing animals and aquaculture in Norway. The table also shows the categorization of the antimicrobial agents as critically important, highly important, and important based on the definitions from WHO. These data have been collected from the databases of Norwegian Medicines Agency. Information on the use of antimicrobials and analogues used in production of vegetables and fruits in Norway was not found.

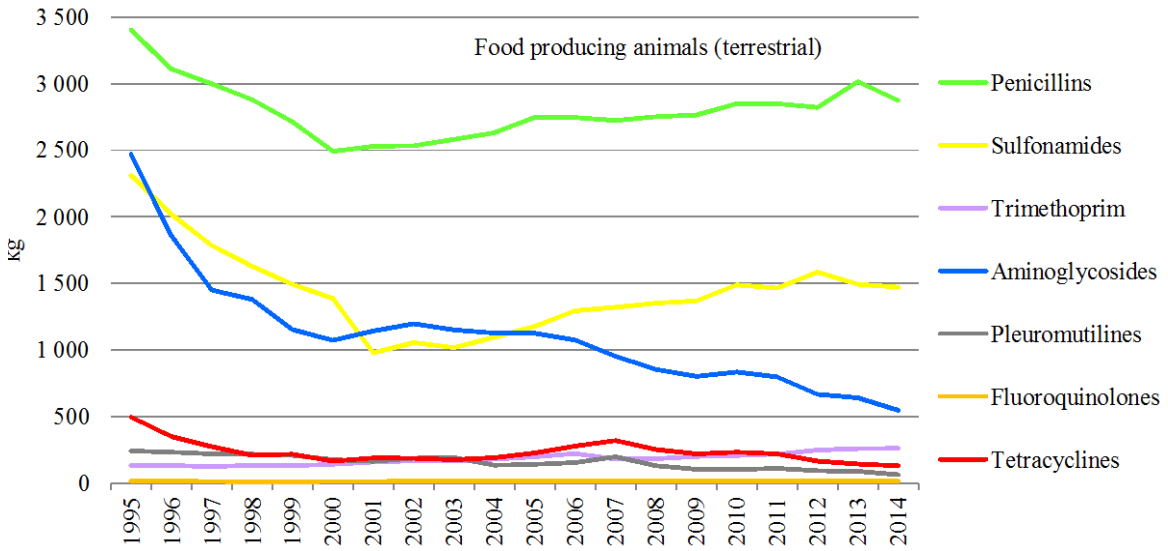


Figure 2-1. Sales in Norway (kilograms active substance) of antimicrobial veterinary medicinal products (VMP) mainly for therapeutic use in food-producing animals for the years 1995-2014 (farmed fish not included). In addition, minor amounts of amphenicols (range 17-27 kg) were sold in 2008-2014 and macrolides (range 0.2-18 kg) during 1995-2014.

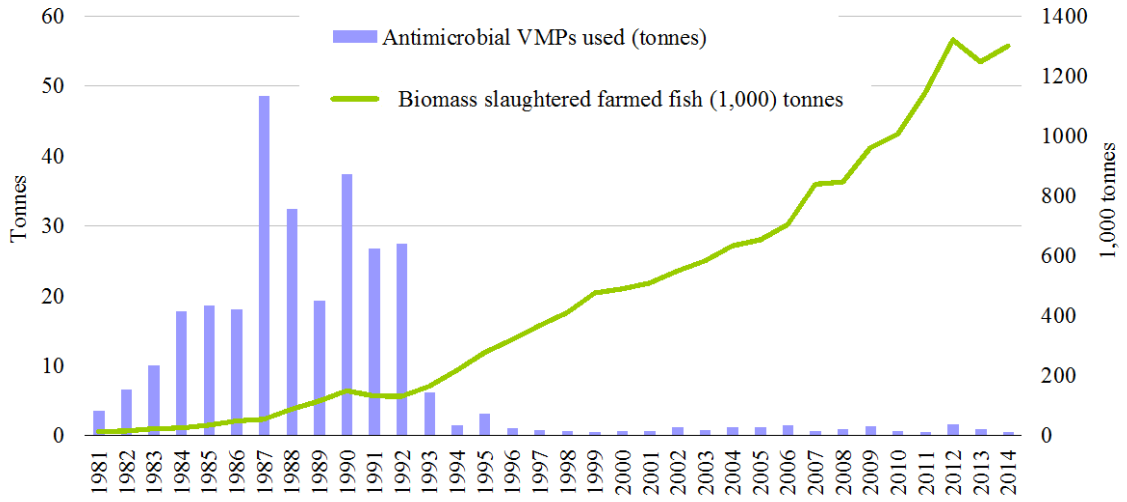


Figure 2-2 Total sales, in kilograms active substance, of antimicrobial VMP for the therapeutic use in farmed fish (1981-2014) versus produced biomass (slaughtered) farmed fish (NORM/NORM-VET, 2013).

Figure 2-1 and Figure 2-2 show the use of antimicrobial agents in food-producing animals and aquaculture in Norway. The use of antimicrobial agents (excluding antifungal agents, which do not induce resistance in bacteria) in plant agriculture is not approved in Norway.

2.4 Summary

The amount of antimicrobial agents used for therapeutic purposes in food-producing animals in Norway is low compared with the amounts in other European countries. In particular, antimicrobial use in fish is very low, due both to a very restrictive attitude towards treatment and but also as a result o effective vaccination against the major bacterial diseases of salmon and rainbow trout. Use of antimicrobial agents in plants, other than azoles that have no ability to induce resistance in bacteria, is not allowed in Norway. Coccidiostatic agents (e.g., narasin) have been evaluated in a separate assessment (www.vkm.no, 2015).

Different antimicrobial agents used in food-producing animals in Norway are compared in Table 12-2 (Appendix I – tables) with antimicrobials listed as proposed by WHO (Collignon et al., 2009):

- Critically important antimicrobials (all antimicrobial agents designated by d),
- Highly important antimicrobials (all antimicrobial agents designated by e),
- Important antimicrobials (no antimicrobial agents were identified), however all antimicrobial agents that are not categorised as d or e should be included in this category.

3 Hazard identification

Hazard identification is implicit in the title of this report and in the terms of reference (ToR).

The issue of AMR in food is addressed either as a direct hazard or as an indirect hazard through resistance transfer (EFSA, 2008).

- The direct hazard is the presence on food of an antimicrobial resistant pathogenic bacterium that can colonise or infect a human after ingestion of the food, or as a hazard that arises if a person acquires the infection through handling contaminated food.
- The indirect hazard arises through resistance transfer, and is defined as an antimicrobial resistant bacterium that may transfer resistance genes to a bacterium pathogenic for humans, either directly or via another commensal bacterium. In this case, the hazard is the resistance gene.

4 Hazard characterisation

4.1 Theoretical background

4.1.1 Modes of action of antimicrobial agents

Different antimicrobial agents have different modes of action that follow one or several of the following pathways (Figure 4-1):

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.

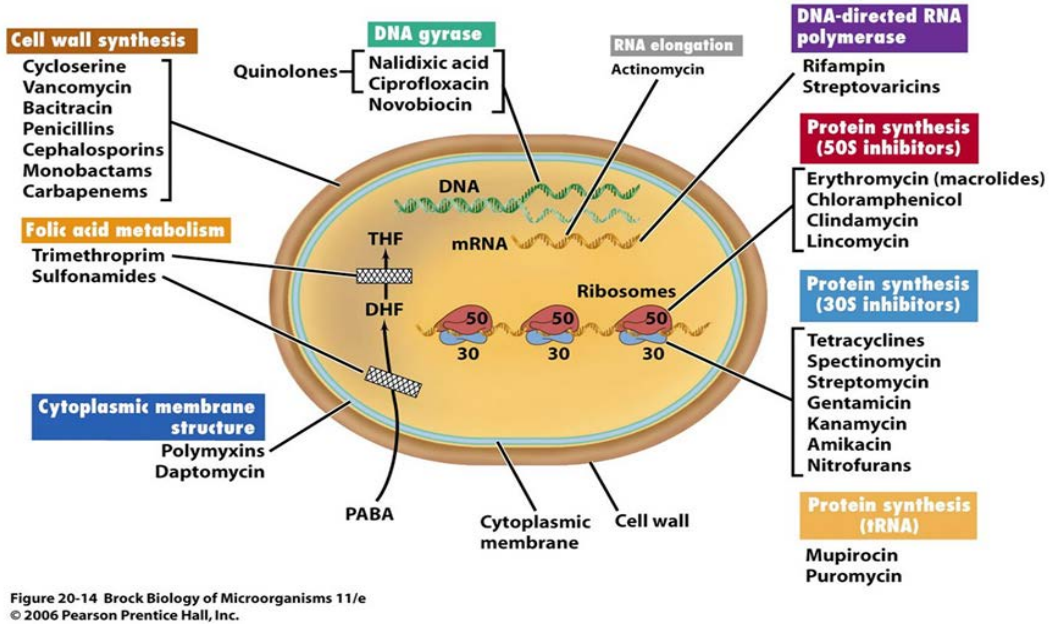


Figure 4-1. The targets for commonly used antimicrobial agents. PABA: Para-aminobenzoic acid; DHF: Dihydrofolate ; THF; Tetrahydrofolate (Madigan and Martinko, 2006)

4.1.2 Resistance mechanisms

Disease-causing bacteria can be described as being clinically resistant if they have a low probability of responding to a drug, even if the maximum dose of antimicrobial agent is administered (Eucast, 2000). Degrees of susceptibility in bacteria are often defined in terms of the minimum inhibitory concentration (MIC) of an antimicrobial agent to prevent bacterial growth, and bacteria are defined resistant to an antimicrobial agent, when the MIC is higher than that of its wild type counterpart.

Bacteria can become resistant to antimicrobial agents by using one or several of the pathways listed under and illustrated in Figure 4-2.

a. Change in the bacterial cell wall permeability.
b. Use of efflux pumps.
c. Antimicrobial target modification.
d. Enzymatic degradation/inactivation of antimicrobials.
e. Alternative pathways.

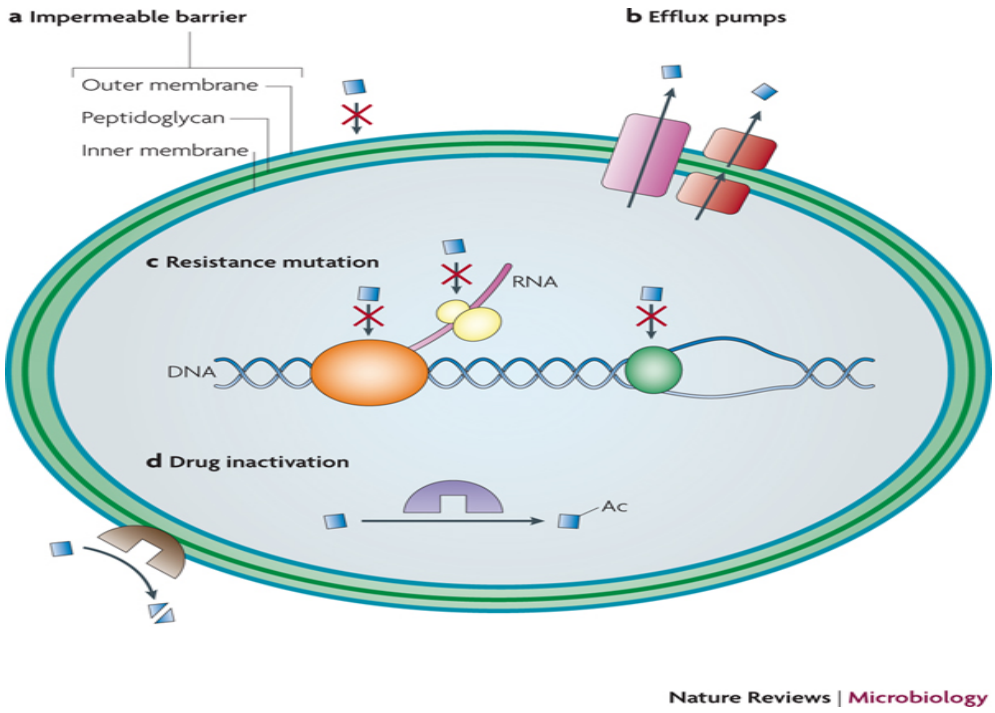


Figure 4-2. Different bacterial resistance mechanisms. Ac: Acetyl group (Allen et al., 2010)

While AMR properties in bacteria are transferred from one generation to the next by vertical gene transfer within same bacterial species, horizontal gene transfer (HGT) may occur both within the same species and between different bacterial species, including unrelated bacterial species.

4.1.3 Horizontal gene transfer (HGT)

HGT may occur within and between bacterial species by conjugation, transformation, or transduction, as has been described extensively in a review article by Huddleston (2014) and illustrated in Figure 4-3.

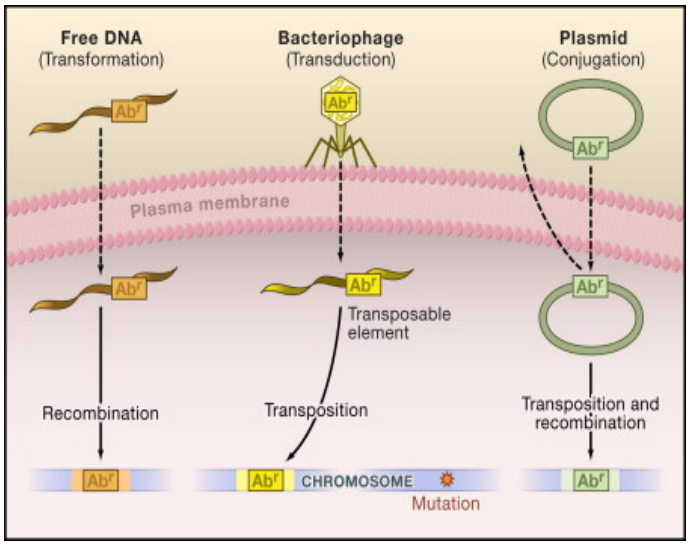


Figure 4-3. Different mechanisms involved in HGT. (Foxman, 2012)

Multiple resistance in bacteria may occur by either co-resistance or cross-resistance (see glossary).

The human and animal gastrointestinal tracts are reservoirs of bacteria of enormous density and species diversity, as well as reservoirs for hundreds to thousands of known AMR genes with the mechanisms in place for horizontal transfer of any gene (Huddleston, 2014).

4.1.4 Drivers for AMR

All use of antimicrobials in human and veterinary medicine, including aquaculture and agriculture, are important drivers for the development of AMR in bacteria.

The spread of AMR does not necessarily respect phylogenetic or ecological borders. Resistance to a certain antimicrobial agent can be selected, even by the use of other agents like antimicrobials, sanitizers, and some metal-containing compounds. The mobility of these AMR genes is attributed to their residence on mobile genetic elements – plasmids, transposons, and integrons (IFT, 2006).

4.2 Surveillance of AMR in food-producing animals in Norway; NORM-VET

The NORM-VET monitoring programme for AMR in the veterinary and food-production sectors was established in year 2000 and is coordinated by the Norwegian Zoonosis Centre at the Norwegian Veterinary Institute. The programme monitors AMR among zoonotic bacteria, such as *Salmonella* spp. and *Campylobacter* spp., indicator bacteria, and clinical isolates submitted to the Norwegian Veterinary Institute. The sample size can only be influenced for indicator bacteria, since monitoring of these bacteria is active. The monitoring of zoonotic bacteria provides a reflection of the actual burden of these pathogens within the animal populations in Norway, and sampling of clinical isolates is mainly passive (depending on the disease situation and the submission of samples to the Norwegian Veterinary Institute by farmers and veterinarians).

In the NORM-VET programme, the prevalence of AMR has been classified in accordance with the levels presented in The European Union Summary Report on AMR in zoonotic and indicator bacteria from humans, animals, and food in 2012 (EFSA, 2014) as follows:

- Rare: <0.1%
- Very Low: 0.1% to 1%
- Low: >1% to 10%
- Moderate: >10% to20%
- High: >20% to 50%
- Very high: >50% to 70%
- Extremely high: >70%

The prevalence of AMR among certain bacteria of the normal enteric microbiota can serve as an indicator for the selective antimicrobial pressure in various populations. These bacteria may form a reservoir of transferable resistance genes from which AMR can spread to other bacteria, including those responsible for disease in animals or humans. Thus, monitoring resistance among indicator bacteria of the normal enteric microbiota from healthy animals, as well as from feed and food, is important in order to obtain an overview of the resistance situation, detect trends, and evaluate the effects of interventions. In NORM-VET, *E. coli* and *Enterococcus* spp. are used as indicator bacteria. The results are published annually in a joint report, together with the results from monitoring of AMR in human pathogens (NORM).

From 2014 and onwards, monitoring among the EU member states (including EEA-member states) has been harmonised by EFSA. This means that the occurrence of AMR in animals and products of animal origin will be more readily comparable in the future.

Substances included in the test panels, as well as some of the epidemiological cut-off values applied in NORM-VET, have been changed over the years. This means that comparisons and

searching for trends is difficult. Only substances that were monitored in 2014 are presented in the Appendix I – tables. Some previously monitored substances belonging to the same class are also included, if comparison is justified.

NORM-VET 2015 includes resistance testing of the indicator bacteria *E. coli* from both cattle and pigs. Samples originate from animals, i.e., faecal samples, and from meat. Additionally samples from feed for cattle and pigs are analysed. Preliminary results indicate that the prevalence of resistance among *E. coli* isolates from cattle and pigs are similar to previous years. However, both quinolone resistance and ESBL/AmpC have been detected in a few isolates from both pigs and cattle (Norwegian Veterinary Institute, personal communication).

4.3 Emerging antimicrobial resistant bacteria

One challenge when assessing the risk of AMR in food chains, is that only a small fraction (approx. 1 %) of the environmental bacteria is culturable, and this places a considerable limit on our knowledge about the true diversity and composition of this reservoir (Finley et al., 2013). This aspect is addressed in the opinion paper by Berendonk et al. (2015), which emphasizes that "current risk assessment models are inadequate to evaluate the effect of antimicrobials and antimicrobial resistance genes on resistance emergence and selection, especially in non-clinical environments". Based on these limitations, VKM has chosen to focus on some specific resistant bacterial species that have emerged at the animal-human interfaces during recent decades. In particular, High Risk Clones of these species seem to have the propensity for epidemic spread and are able to establish themselves in both animals and humans. The choice is based on zoonotic potential, pathogenicity, and limited alternatives for treatment of infections caused by these bacteria.

4.3.1 Vancomycin-resistant Enterococci (VRE)

Resistance to the glycopeptide vancomycin emerged in enterococci (primarily *Enterococcus faecium*) in the late 1980s in both Europe and the United States. Considerable amounts of vancomycin had been used as vancomycin was a last-resort drug for infections of multi-resistant staphylococci and enterococci, particularly in US hospitals, and VRE became an important nosocomial pathogen. In Europe, an environmental reservoir of VRE was documented due to the use of the glycopeptide antimicrobial avoparcin to promote growth in food animals. *E. faecium* is highly clonal in its structure, and clones that are typical and adapted to patients, healthy people, and animals are evolutionarily distinct. Nine different types of vancomycin-resistance gene clusters have been characterized in enterococci to date. The *vanA* cluster is the most prevalent type, and is carried on transposon Tn*1546*, which is transferable by conjugation. However, considerable heterogeneity exists among Tn*1546* elements due to insertions and deletions of gene sequences. Large *van*A-carrying plasmids have been found in both pig and human VRE isolates (*E. faecium*), and the same Tn*1546*

type has been found in clonally unrelated poultry, pig, and human strains (Biavasco et al., 2007). Thus, a common Tn*1546* reservoir is probably accessible to a variety of *E. faecium* recipients by HGT. In contrast to that which has been found in poultry, some researchers have emphasized similarities between *van*A-elements from enterococci from pigs and humans (Willems et al., 2000; Willems et al., 2005).

4.3.2 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is an important pathogen for humans and for many animal species, and MRSA is currently a leading cause of hospital-acquired and community-acquired infections worldwide. MRSA is resistant to all beta-lactams, which reduces the therapeutic options considerably. Semi-synthetic penicillins, such as methicillin, were introduced in the late 1950s as a response to the rapid development of penicillinase-producing *S. aureus*. Subsequently, a wave of hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) strains emerged. From the mid-1990s and onwards, MRSA with novel properties invaded the community. This community-acquired MRSA (CA-MRSA) combined rapid spreading ability with resistance to antimicrobial agents. Livestock-associated MRSA (LA-MRSA) were detected for the first time a decade ago but are now globally distributed. LA-MRSA seem to have the best colonization ability in pigs, however, this MRSA variant has also been detected in samples from cattle, sheep, poultry, horses, and companion animals (Vanderhaeghen et al., 2010). LA-MRSA developed in the animal reservoir from a human MSSA (methicillin-susceptible *S. aureus*) strain, and the prominent clonal complex, CC398, was, until recently, regarded as having limited ability to re-establish itself in the human reservoir. Currently, LA-MRSA is prevalent in certain high-risk groups of workers in direct contact with live animals. However, LA-MRSA is also sometimes detected in humans without any known exposure to pigs, and the colonization and transmission dynamics of LA-MRSA in humans are not completely understood. MRSA continues to be a major threat to public health. CA-MRSA and LA-MRSA have therefore become a challenge for some countries that, until now, had maintained low rates of MRSA in the human population (Stefani et al., 2012).

Through HGT, MRSA have acquired a gene called *mecA* (or *mecC*). The *mecA/mecC* gene is located on a complex mobile genetic element, named the staphylococcal chromosomal cassette, SCC*mec,* which was probably acquired from coagulase-negative staphylococci (CoNS) (Hanssen and Ericson Sollid, 2006). In addition, SCC*mec* elements contain regulatory elements, recombinase genes, and may contain genes encoding resistance to other antimicrobial agents. SCC*mec* elements may be disseminated between staphylococcal species, but, to date, this element has not been detected in bacterial species other than staphylococci.

4.3.3 Quinolone-resistant (QR) Gram-negative bacteria

Quinolone and fluoroquinolone antimicrobials are potent, broad-spectrum antimicrobial agents commonly used to treat a range of infections. Resistance to these agents is mainly introduced by chromosomal mutations in the genes that encode the drug target enzymes. Resistance can increase via production of multidrug-resistance efflux pumps, modifying enzymes, and/or target-protection proteins, or combinations of these. Resistance towards nalidixic acid requires only one mutation, while resistance towards the more broad-spectrum fluoroquinolones needs two or more mutations. Genes encoding for quinolone resistance are mainly located on chromosomes and may be spread to other bacteria via HGT. Different transferable mechanisms of quinolone resistance have been described, mainly attributed to plasmids (plasmid-mediated quinolone resistance, PMQR). These resistance mechanisms usually result only in a slight increase in the MIC of quinolones, but they possess an additive effect and may facilitate the acquisition of full quinolone resistance (Ruiz et al., 2012).

Fluoroquinolone-resistant clinical isolates of bacteria have emerged and data show that resistance to this class of antimicrobials can have diverse, species-dependent impacts on host-strain fitness (Redgrave et al., 2014). A high prevalence of resistance has been observed among *E. coli* and *C. jejuni.* In *E. coli*, fluoroquinolone resistance may be associated with multi-resistance. Particular emphasis is on the High Risk Clone *E. coli* ST131, which usually also contains the CTX-M-15 ESBL-determinant in addition to fluoroquinolone resistance (see below). In Norway, there has been a considerable increase in resistance to fluoroquinolones in *E. coli* causing bloodstream infections (BSI) and urinary tract infections (UTI) during the last decade (NORM/NORM-VET 2013). In several European countries it has been demonstrated that poultry have a high prevalence of quinolone-resistant *E. coli* in the intestinal flora (EFSA, 2014). Such resistant *E. coli* have also been detected from healthy animals in Norwegian poultry production (NORM/NORM-VET, 2014). Quinolone-resistant *C. jejuni* has been found in several animal reservoirs, such as poultry, pigs, and cattle.

4.3.4 Extended-spectrum Beta-Lactamase (ESBL/pAmpC)-producing bacteria

Resistance in Gram-negative bacteria to extended-spectrum cephalosporins, like cefuroxime, ceftazedime, and cefotaxime, has been developing over two decades. It is most often caused by extended-spectrum β-lactamases (ESBLs) (class A, or $ESBL_A$), but may also be conferred by plasmid-mediated AmpC-type enzymes (Class C, called $ESBL_M$ or pAmpC). Hyper-production of AmpC-type enzymes due to chromosomal mutations can mediate resistance to cephalsosporins. Plasmids that harbour ESBL and/or pAmpC genes may also carry other resistance genes, meaning that ESBL/pAmpC-producing pathogens can also be resistant to other classes of antimicrobial agents (MacVane et al., 2014). *Enterobacteriaceae* is the main bacterial family associated with ESBL/pAmpC production, of which *E. coli* and

Klebsiella spp. are most important. Expansion of genes encoding ESBL/pAmpC enzymes can occur either by emerging bacterial clones or by HGT due to the spread of plasmids between bacteria of the same and/or different species (Brolund et al., 2014).

The High Risk Clone *E. coli* ST131 has expanded rapidly in the human bacterial population. This clone has been found to a limited degree in poultry, and even more seldom in pork (Manges et al., 2015). Reasons for the successful dissemination and expansion may include increased transmissibility, greater ability to colonize and/or persist in the intestine or urinary tract, enhanced virulence, and more-extensive AMR compared with other *E. coli* (Banerjee and Johnson, 2014).

4.3.5 Carbapenemase-producing (CP) bacteria

Carbapenemases are another emerging mechanism for resistance to β-lactams; these cause resistance to carbapenems as well as other β-lactams (class B) (Nordmann, 2014). Carbapenem resistance is commonly associated with combined resistance to 3rd-generation cephalosporins, aminoglycosides, and fluoroquinolones (ECDC, 2012). Carbapenemases are biochemically diverse, and it is also increasingly evident that carbapenem-resistance can be conferred through other mechanisms, like AmpC enzymes and beta-lactamases, in combination with mechanisms that limit carbapenem entry into bacterial cells (Sartelli et al., 2014). Therapy options are limited for patients with infections caused by beta-lactam-resistant pathogens, particularly those that are resistant to carbapenems, and there are significant limitations to the few existing alternatives to carbapenems. In a recently published paper from Egypt, high rates of ESBL/AmpCs and carbapenemases (65 % and 11.3 %, respectively) were detected in *Enterobacteriaceae* isolated from retail chicken meat (Abdallah et al., 2015).

For detection of cabapenemase-producing Gram-negative bacteria, selective agar media containing carbapenem has been used from 2015. So far, carbapenemase-producing Gram-negative bacteria, belonging to *Enterobacteriaceae,* have not been reported in food-producing animals in Norway (NFSA, personal communication).

It has been documented that bacteria belonging to the *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter* spp., and *Myroides* spp., isolated from seafood, may be carbapenemase-producing bacteria (Morrison and Rubin, 2015). Such bacterial species will evade detection by surveillance programmes that are based on detection of traditional indicator bacteria and known pathogens.

Multi-drug resistant *Enterobacteriaceae*, mostly among *E. coli* and *Klebsiella pneumonia*, with resistance to carbapenem conferred by New Dehli metallo-ß-lactamase 1 (NMD-1) are potentially a major global human health problem. NDM-1 isolates are highly resistant to all antibiotics except to tygecycline and colistin (polymyxin) (Kumarasamy et al., 2010).

Until now, colistin resistance has occurred via chromosomal mutations and, although clonal outbreaks have been reported, the resistance is often unstable, imposes a fitness cost upon the bacterium and is incapable of spreading to other bacteria (Falagas et al., 2011). A recently published paper (Liu et al., 2015) from China reported a major increase of colistin resistance in commensal *E. coli* from food animals in China. The authors found that an *E. coli* strain isolated from a pig, possessing colistin resistance that could be transferred to another strain. The study resulted in finding the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in *Enterobacteriaceae*. Bacteria belong to *Enterobacteriacae* isolated from food and human containing *mcr-1* gene has been reported from Denmark (DTU, 2015).

4.4 Resistance in zoonotic pathogens

During the last decade, classical zoonotic pathogens such as special variants of *E. coli*, *Salmonella* spp., other *Enterobacteriaceae,* and *Campylobacter* spp., have been associated with increased rates of AMR. Resistant variants of these bacteria may reduce the available therapeutic options if antimicrobial therapy is needed (EFSA/ECDC, 2013).

Occurrence of AMR in zoonotic pathogens isolated from clinical samples in Norway is not well documented due to under-reporting of cases and the current routines for sample testing.

4.4.1 Zoonotic *E. coli*

Most of the pathogenic variants of *E. coli* are not regarded as zoonotic pathogens, with some shigatoxin-producing *E. coli* (STEC) and atypical enteropathogenic *E. coli* (aEPEC) being important exceptions. Cattle and sheep are the most important animal reservoirs of STEC and aEPEC, and these bacteria are subject to ecological processes that create and select AMR strains in their reservoirs, similarly to other zoonotic pathogens. In general, *E. coli* can harbour resistance genes towards a broad spectrum of antimicrobials, often in combinations that result in multi-resistance.

4.4.2 *Salmonella* spp.

The *Salmonella* spp. data presented in EFSA/ECDC report (2015) comprise results (see Appendix II - Figures) for all reported non-typhoidal *Salmonella* serovars that have been combined to represent the overall occurrence of AMR in *Salmonella* in humans and the various animal and food categories. These data show that high proportions of human *Salmonella* spp. isolates were resistant to ampicillin (36.1 %), sulphonamides (35.7 %), and tetracyclines (34.5 %), and that multi-resistance was common (31.8 %), with very high occurrence for some serovars and in some countries. The proportion of *Salmonella* spp. isolates resistant to either of the clinically important antimicrobials ciprofloxacin and cefotaxime was, on average, relatively low, but an exception was an extremely high

proportion of *S*. Kentucky that was resistant to ciprofloxacin. In total, 44.2 % of all isolates tested were susceptible to the complete range of antimicrobial classes in the human data collection.

Resistance of *Salmonella* spp. isolates from meat to tetracyclines, ampicillin, and sulphonamides typically ranged from moderate to extremely high. The highest levels of resistance to ciprofloxacin and nalidixic acid were noted in isolates from meat from broilers and turkeys.

Among *Salmonella* spp. isolates from animals, most Member States reported moderate or high to extremely high resistance to tetracyclines and sulphonamides, and similar or slightly lower levels of ampicillin resistance. Resistance levels were generally higher in isolates from pigs and turkeys than from broilers, laying hens, breeding hens, and cattle. High to extremely high levels of resistance to ciprofloxacin and nalidixic acid were observed in *Salmonella* spp. isolates from fattening turkeys and broilers compared with the low or moderate levels recorded in *Salmonella* spp. isolates from laying hens, pigs, and cattle.

Data are presented in Figure 13-1 and Figure 13-2.

The situation regarding occurrence of *Salmonella* spp. in food-producing animals in Norway is very good, as the animal populations are considered virtually free from *Salmonella*. In order to document and maintain this favourable situation, Norway runs an extensive surveillance programme covering both live animals (cattle, pigs, and poultry) and meat samples. The *Salmonella* isolates examined in NORM-VET include those detected in this programme, as well as those detected by clinical submissions to the Norwegian Veterinary Institute. In 2014, a total of 24 *Salmonella* isolates from animals were susceptibility tested, of which 16 belonged to *S*. Typhimurium. Of the 24 isolates, 13 were fully susceptible, six isolates were resistant to one antimicrobial, one to two antimicrobials, three to three antimicrobials, and one isolate, originating from a cattle herd, was multi-resistant to five antimicrobials. Another isolate originating from a chicken farm was multi-resistant to seven of the antimicrobial substances tested (NORM/NORM-VET, 2014).

4.4.3 *Campylobacter* spp.

EU surveillance data on *C. jejuni* and *C. coli* isolates from humans (EFSA/ECDC, 2011; EFSA/ECDC, 2012; EFSA/ECDC, 2013) showed no major changes in overall resistance during three years of surveillance. *C. coli* was more resistant to most antimicrobials than *C. jejuni*, including erythromycin, ciprofloxacin, nalidixic acid, and tetracycline, but not ampicillin. The EU summary report in 2013 also contained information on *Campylobacter* isolates from animals and food reported by 17 Member States and 2 non-Member States (Norway and Switzerland) in 2011. For *C. jejuni* isolates from broilers, the occurrence of resistance to ciprofloxacin, nalidixic acid, and tetracycline was high, while resistance was low for

erythromycin and gentamicin. In comparison, among *C. coli* isolates from broilers, resistance to ciprofloxacin, nalidixic acid, and tetracycline was extremely high. Levels of resistance to erythromycin and gentamicin were moderate and low, respectively. Some Member States showed statistically significant trends of increasing resistance of broiler isolates to ciprofloxacin and nalidixic acid (EFSA/ECDC, 2013).

In Norway, the isolates of *C. jejuni* in broilers included in NORM-VET originate from caecal samples collected by the "The surveillance programme for *Campylobacter* spp. in broiler flocks". The aim of the surveillance programme is to reduce human exposure to *Campylobacter* spp. through broiler meat. All broiler flocks slaughtered before 50 days of age are tested for *Campylobacter* spp. In 2013, one *C. jejuni* isolate per positive flock was submitted for susceptibility testing (96 isolates). The prevalence of AMR is low, as 90.6 % of the isolates tested were susceptible to all antimicrobial agents tested. Resistance to one or two antimicrobial agent was detected in 3.1 % and 6.2 % of the isolates, respectively. The therapeutic use of antimicrobial agents in broilers is very low and the products for such use contain either amoxicillin or phenoxymethylpenicillin. Commercial poultry is treated against bacterial infections by adding the antibacterial VMP through drinking water. Nalidixic acid is not used in poultry. The prevalence of resistance to ciprofloxacin seems to have increased in recent years, rising from 1.0 % in 2007, to 4.2 % in 2011, to 5.2 % in 2013. However, these changes are not significant, and future monitoring is needed to determine whether this is a continuing trend.

4.4.4 *Listeria* spp.

L. monocytogenes rarely develops acquired resistance to antimicrobial agents and there are no data on the prevalence of AMR in *Listeria* spp. in the EFSA/ECDC reports (Bertsch et al., 2014; Granier et al., 2011). However, several individual studies have reported antimicrobial resistant strains of *L. monocytogenes* isolated from food and food-processing areas. For clinical isolates, AMR probably remains a marginal phenomenon. One study concluded that acquired resistance in clinical isolates of *L. monocytogenes* is of no clinical consequence as it does not concern the first-line treatment for listeriosis (Morvan et al., 2010).

Norwegian surveillance data for AMR in *Listeria* spp. are not available in NORM/NORM-VET reports. We are also not aware of published papers on antimicrobial resistant *Listeria* in Norway (Bowker et al., 2012).

4.5 Food chains as a source of antimicrobial resistant bacteria

Food consumption, contact with raw or processed food during food processing or preparation, and direct contact with animals or their faeces may serve as routes for the transmission of antimicrobial resistant bacteria from animals to humans, reflecting the link

between human and veterinary medicine as described in the “One Health Initiative”. There are several routes for transfer of AMR from animal production to humans and vice versa, as shown in Figure 4-4.

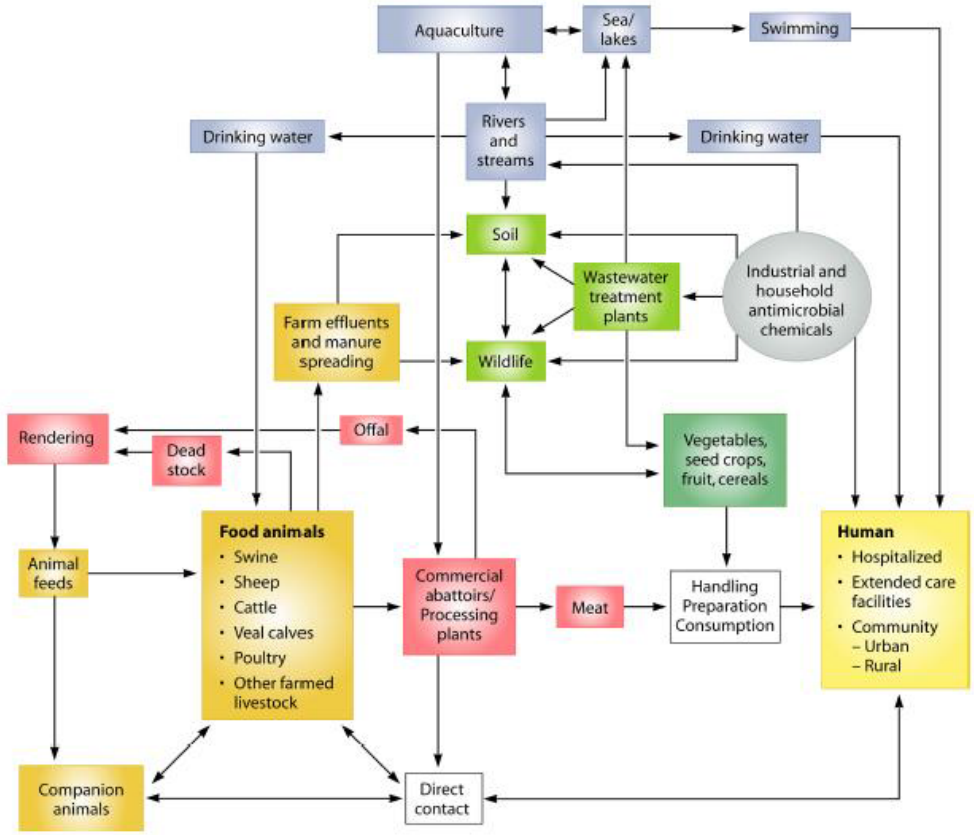


Figure 4-4. Dissemination of antimicrobials and AMR within agriculture, communities, hospitals, wastewater treatment, and associated environments (Davies and Davies, 2010).

One of the routes includes direct contact between animals and farm workers. Several studies have found genetic similarities between resistant *E. coli* strains isolated from animal production and strains isolated from farm workers (de Been et al., 2014; Dohmen et al., 2015; Hammerum et al., 2014; Lazarus et al., 2015; van den Bogaard et al., 2001; van den Bogaard et al., 2002). Similarly, transfer of methicillin-resistant *S. aureus* (MRSA) from pigs to farmers (and others with work-related exposure to pigs) has been described (Feingold et al., 2012; Huijsdens et al., 2006).

Another important route of resistance transfer from carrier animals to humans concerns contamination through food processing, particularly meat. One critical step is the slaughter and evisceration (removal of guts) process. The surfaces of meat and meat products can be contaminated by faecal flora during the slaughter process, and this flora may contain

resistant bacteria that are able to cause infection in humans, to colonize the human intestinal tract, and/or transfer their resistance genes to other resident bacteria.

Food that is not usually cooked or heat-treated, such as some fruits and vegetables, are of special importance concerning food consumption. For example, faecally contaminated vegetables have been linked to several microbial foodborne outbreaks where the causative strain or accompanying indicator bacteria carried resistance genes. Another route of resistance transfer involves soil habitats; soil is the receiving environment for faecal matter disposed of by animal farms and that may contain antimicrobial resistant bacteria. Some types of microorganisms can survive for prolonged periods in biofilms or in dust. They can occur on different materials and vehicles, or in animals, food, plants, and feed, and can transfer from one environment to another by travel or transport. The environmental aspects have recently been addressed in an opinion paper by Berendonk et al. (2015). The authors state: “At present, it is not clear to what extent environmental antimicrobial resistant bacteria and AMR genes promote the acquisition and spread of AMR among clinically relevant bacteria, or whether AMR genes that are acquired by both clinically relevant bacteria and strictly environmental bacteria originate from the same reservoirs”.

Import

In this assessment, the probabilities associated with antimicrobial resistant bacteria/AMR genes in imported food-producing animals, meat, dairy products, seafood, fresh produce (fruit, berries, and vegetables) and genetically modified organisms have not been assessed, since it was not a part of the mandate of this risk assessment.

4.5.1 Pigs

In Norway, resistance data for bacteria from pigs have been generated since the start of the NORM-VET programme in year 2000. Bacterial species investigated have been: *E. coli* from the intestinal flora of healthy animals (indicator bacteria), *E. coli* from pork (indicator bacteria), and ESBL/AmpC-producing *E. coli* (using a selective method on faecal samples). In addition, investigations for MRSA have been carried out. Materials investigated have been nasal swabs, samples from skin surfaces, and environmental samples. All MRSA investigations have been carried out using a selective method recommended by EFSA (EFSA, 2012).

In general, the monitoring data show a low occurrence of resistance in indicator *E. coli* from healthy animals and from pork (Table 12-4). However, some multi-resistant isolates have been detected. These isolates usually exhibited resistance against the “older” antimicrobial agents like streptomycin, sulphonamides, ampicillin, and tetracycline. Resistance to fluoroquinolones was very rare. Screening for ESBL/AmpC-producing bacteria has been performed twice; in 2009 and 2011 (NORM/NORM-VET, 2009; NORM/NORM-VET, 2011).

Only one ESBL/AmpC-positive finding has been recorded (NORM/NORM-VET, 2011), indicating a low prevalence of ESBL/AmpC-producing *E. coli* in the Norwegian pig population. The ESBL/AmpC-encoding bla_{TEM-52} was responsible for resistance to extended-spectrum cephalosporins in this isolate.

Screening for MRSA in pigs has been conducted four times. The first screening was performed in 2008 (EU MRSA baseline study) and LA-MRSA was not detected in pig herds investigated in Norway (EFSA, 2010). However, MRSA ST8 (MRSA variant of human origin) was detected in one herd (Sunde et al., 2011b).

LA-MRSA was found in samples from pigs in Norway for the first time in 2011. The NORM-VET programme identified MRSA in pigs sampled in one (out of 11) abattoir. However, in 2013 the Norwegian Veterinary Institute diagnosed an infection with LA-MRSA (*spa*-type t034) in a pig sent for autopsy from a farm raising fattening pigs. Follow-up testing showed that the infection could be traced back to a large supplier of piglets. During the summer of 2013, a person working on a farm with fattening pigs in the southwest of Norway was diagnosed with a severe infection caused by LA-MRSA. The pigs in both this farm and the connected breeder unit were colonized with LA-MRSA. Follow-up testing of all pig herds with contact by animal trade with the two positive breeder units resulted in several findings of LA-MRSA (Espetvedt et al., 2014). The finding of LA-MRSA in these two networks in 2013, connected via trade of live pigs, initiated follow-up sampling of animals and farm environments in the networks, as well as screening of persons at positive farms (and other persons exposed to positive animals, such as workers in slaughterhouses etc.). In total, 24 LA-MRSA positive herds were found and at least 32 persons that had been in contact with animals on positive farms were found to be carriers of LA-MRSA. In order to obtain an overview of the MRSA prevalence in Norwegian pig production, an extensive MRSA screening of 988 pig herds (mainly production units) was performed during spring 2014; one positive pig herd was identified (www.mattilsynet.no). The LA-MRSA strain found in the first two events had a multi-resistant profile, as it was resistant to several other antimicrobial agents including fluoroquinolones, tetracycline, clindamycin, and some strains were also resistant to erythromycin. The multi-resistant nature of this LA-MRSA contributed to the decision to try to eradicate MRSA from positive herds in order to avoid establishment of reservoirs for further transmission between farms and from pigs to humans.

Despite these efforts, LA-MRSA was detected in additional pig herds during 2015. These findings initiated contact-tracing and follow-up testing of a considerable number of pig farms and people associated with positive farms. It is believed that MRSA was introduced via foreign workers, and there is a considerable challenge concerning control and prevention of introduction of LA-MRSA to the agricultural sector in Norway. Eradication of LA-MRSA on positive farms has been attempted and the overall goal is to keep the Norwegian pig industry free from LA-MRSA.

4.5.2 Poultry

Bacteria from poultry have been included in the NORM-VET programme since the start of the programme in year 2000. Indicator bacteria from the intestinal flora of healthy animals (*E. coli* and *Enterococcus* spp.) and *E. coli* from cases of septicaemia (broilers) have been tested. In addition, screening for ESBL/AmpC-producing *E. coli,* quinolone-resistant *E. coli,* and VRE have been performed using selective methods. Detailed descriptions of the selective methods used by NORM-VET are provided in the respective annual reports. The compiled resistance data from indicator *E. coli* are shown in Table 12-5 (broiler) and Table 12-6 (turkey). The data indicate a high occurrence of resistance in *E. coli*. Indicator isolates are included by random selection of one *E. coli* isolate from each sample included in the monitoring programme.

In 2006, the first ESBL/AmpC-producing *E. coli* strain was detected from the intestinal flora of a healthy broiler in Norway (NORM/NORM-VET, 2006). This finding could be interpreted as an "early warning" that poultry could be associated with ESBL/AmpC-producing *E. coli.* The genetic background for ESBL/AmpC-production was the presence of the bla_{TEM-20} gene (Sunde et al., 2009). In the 2011 NORM-VET programme, a selective method for detection of ESBL/AmpC in faecal samples (boot swabs) from broilers was used for the first time. By using the selective method it was demonstrated that ESBL/AmpCs occurred in 43 % of the broiler flocks investigated (n=252). It was also shown that ESBL/AmpC occurred frequently in poultry meat (chicken fillets) available on the market, as approximately one third of chicken fillets investigated (n=205) contained ESBL/AmpC-positive bacteria (NORM/NORM-VET, 2012). In 2014, the ESBL/AmpC investigations were repeated. Instead of investigating boot swabs, caecal samples from slaughterhouses were investigated. The meat investigations were performed in the same way as in 2012. Preliminary results indicated similar high frequencies of ESBL/AmpC in both categories of samples (NORM/NORM-vet 2014). As with the situation in many other countries, it has now been demonstrated that broiler production in Norway has a high prevalence of *E. coli* that are resistant to 3rd-generation cephalosporins (cefotaxim and/or ceftazidime (Table 12-5)). Poultry production in Norway depends on import of grandparent/parent animals for breeding, as well as hatching eggs. Resistant bacteria have probably been introduced via imports. A Norwegian study has shown that breeding animals imported for broiler production can be carriers of ESBL/AmpC-positive bacteria (Mo et al., 2014). The current findings in NORM-VET using the selective method indicate that there has been a small reduction in the prevalence of ESBL/AmpC-producing *E. coli* in broiler flocks and broiler meat since the investigations in 2011 (43 %) and 2012 (32.2 %), respectively, although the reduction is not statistically significant. In addition, a change in sampling procedure (from boot swabs to pooled caecal samples) may have affected the results. Boot swab sampling mirrors the prevalence in the broiler house, whereas caecal samples indicate the occurrence in the animals. The results from broiler flocks and broiler meat contrast with results reported by the industry that showed that only a

small proportion of imported breeding flocks were positive in 2014. However, the imported breeding flocks were tested at hatching and were too young to be the parenting flocks of the majority of the broiler flocks tested in the NORMVET programme in 2014. Therefore, it is probably too early to observe any effect of the measures implemented by the industry on the prevalence of AMR.

It has been demonstrated that the prevalence of quinolone-resistant *E. coli* in turkey meat produced in Norway is considerable, as nearly 50 % of the meat samples investigated contained such bacteria (NORM/NORM-VET, 2013). Similar investigations have recently been performed with meat and faecal samples from chicken. By using a selective method, quinolone-resistant *E. coli* was found in 89.5 % and 70.7 % of broiler caecal samples and broiler meat samples, respectively. The majority of the isolates were only resistant to the quinolones nalidixic acid and ciprofloxacin, although resistance to one additional class of antimicrobial agent was observed in 12.8 % and 18.6 %; and to two or more in 10.7 % and 9.3 % of the isolates, respectively. Most quinolone-resistant isolates had MIC profiles indicating that the phenotype was probably mediated by mutations in the bacterial chromosome. Although the selective method detected quinolone-resistant *E. coli* in most of the samples, only a few isolates were usually detected by the non-selective procedure. This indicates that the within-flock prevalence of quinolone-resistant *E. coli* may be low. The findings were somewhat surprising, as there is no selection pressure from quinolone use in Norwegian broiler production. It is unknown when, why, and how this quinolone resistance has emerged in broilers, nor what impact this reservoir may have (NORM/NORM-VET, 2014). A recent study from Sweden demonstrated quinolone-resistant *E. coli* in the broiler-breeding pyramid (Borjesson et al., 2015). It is possible that quinolone-resistant *E. coli* were introduced to Norwegian broiler production via breeding material, in the same way as cephalosporin-resistant *E. coli* was introduced.

The prevalence of resistance to most antimicrobial agents among *Enterococcus* spp. is considered moderate. Compiled data are shown in Table 12-7 (broiler) and Table 12-6 (turkey). By using a selective method it was shown that 16 % of flocks were positive for VRE in 2011. All isolates were identified as *E. faecium* (NORM/NORM-VET, 2011). The prevalence of VRE has fluctuated over the years. This may be a consequence of differences in sampling procedures and types of material investigated (boot swabs vs faecal samples from live birds). In 2013, 12.2 % of samples from turkeys were positive for VRE. All isolates were identified as *E. faecium* containing the *vanA* gene (NORM/NORM-VET, 2013).

4.5.2.1 Eggs

Monitoring of AMR in bacteria originating from eggs has not been included in NORM-VET. Only a few studies worldwide have investigated the occurrence of AMR in *Salmonella* spp. and *E. coli* originating from faecally contaminated eggshells.

4.5.3 Cattle

Resistance testing of indicator bacteria from healthy cattle and beef has been performed in 2003, 2005, and 2010 (*E. coli* and *Enterococcus* spp. from faecal samples and minced beef). Data compiled from testing of indicator bacteria are shown in Table 12-11 and Table 12-12. The data indicate a low occurrence of resistance among indicator bacteria from cattle in Norway. Resistance to fluoroquinolones among *E. coli* has never been detected. ESBL/AmpC screening using a selective method was carried out on samples from cattle in 2015. In September 2015, MRSA was isolated from cattle in a herd that had previously tested positive for MRSA in the farm's pig population (www.mattilsynet.no, 29.09.15).

4.5.3.1 Mastitis

Staphylococci and streptococci are the most prevalent bacteria causing mastitis in cattle and goats in Norway. *S. aureus* from dairy cattle and goat milk were tested in year 2000 (NORM/NORM-VET, 2000). The results from cow's milk showed a low occurrence of resistance, as approximately 5 % of isolates investigated were resistant to penicillin G, streptomycin, and fusidic acid. Very low resistance rates to the other antimicrobial agents were detected as illustrated in Table 4-1. The low levels of penicillin-resistant *S. aureus* from cattle mastitis was confirmed by Garmo et al. (2010), and also by the Norwegian Cattle Health Services that demonstrated that penicillin-resistant *S. aureus* from clinical mastitis in cows was reduced from 16 % in 1994 to 3-4 % in 2014 (Helsetjenesten, 2015). However, both studies also confirm the higher degree of resistance towards penicillin in CoNS; around 40-50 %. There seems to be seasonal variation in the resistant isolates, and there are differences associated with CoNS-species. Cases of bovine mastitis caused by MRSA have not been recorded in Norway to date (Helsetjenesten, 2015; NORM/NORM-VET, 2012)

Only 1 % of the *S. aureus* isolates originating from goat milk were resistant to penicillin G. Resistance to other antimicrobial agents was not detected, as shown in Table 4-2.

Table 4-1 Percentage of resistance in *S. aureus* isolated from cow's milk (bulk tank milk samples) in Norway, 2000

Substance	2000 (n=121)
Tetracycline*	0.8
Chloramphenicol	0
Penicillin G*	4.1
Oxacillin	0
β–lactamase	5.0
Cephalothin	
Trimethoprim	
Sulphonamides	0
Erythromycin	0
Clindamycin	0
Streptomycin	5.8
Gentamicin	0
Enrofloxacin	0
Fucidic acid	5.8

Table 4-2 Percentage of resistance in *S. aureus* isolated from goat milk (bulk tank milk samples) in Norway, 2000

Substance	2000 (n=96)
Tetracycline*	0
Chloramphenicol	0
Penicillin G*	1.0
Oxacillin	0
β–lactamase	1.0
Cephalothin	0
Trimethoprim	0
Sulphonamides	0
Erythromycin	0
Clindamycin	0
Streptomycin	0
Gentamicin	0
Enrofloxacin	0
Fucidic acid	0

4.5.3.2 Milk and dairy products

Pasteurised milk

Due to mandatory regulations, almost all milk for human consumption is pasteurised at the dairy (LOVDATA, 2002). In the dairy industry, pasteurisation is defined as heat treatment of milk at 71.7°C for 15 seconds (Abrahamsen et al., 2003), also called HTST pasteurisation.

Certain dairy products are subject to UHT pasteurisation, where the milk is heated to a much higher temperature for a few seconds, (e.g., 138°C for 2 seconds), resulting in a sterile product. Pasteurisation will have a bactericidal effect on most of the vegetative forms of the bacterial contaminants of raw milk.

E. coli from cattle bulk milk has been included in NORM-VET only once (in 2009) and the data obtained indicated a low resistance prevalence. However, the sample size was small; only 15 isolates underwent susceptibility testing. Among the 15 isolates, one multi-resistant isolate was found exhibiting resistance to ampicillin, sulphonamides, tetracycline, and streptomycin (NORM/NORM-VET, 2009). The results are listed in Table 4-3.

Table 4-3 Percentage of resistance in indicator bacterial isolates (*E. coli*) isolated from cattle (bulk milk samples) in Norway, 2009. Cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available

Substance	2009 (n=15)
Tetracycline*	6.7
Chloramphenicol	0
Florfenicol	0
Ampicillin	6.7
Cefotaxime	0
Ceftazidime	ND
Sulphamethoxazole	6.7
Trimethoprim	0
Gentamycin	0
Streptomycin	6.7
Kanamycin	0
Ciprofloxacin*	0
Nalidixic acid	0
Colistin	ND

*Oxytetracycline was tested instead of tetracycline before 2005, and enrofloxacin instead of ciprofloxacin before 2006

Raw milk

Raw milk is an excellent growth medium for many microorganisms because of its high water content, near neutral pH, and its variety of nutrients (Doyle et al., 2001). A number of environmental, commensal, and pathogenic bacteria may contaminate the milk along the value chain; from animal feed and the environment, from the animal itself (skin, faeces, udder), from the milking process at the farm, and from the processing of milk at the dairy until packaging. The bacteria introduced may be resistant to one or more antimicrobials.

Some of these microorganisms may represent a real threat to human health. A qualitative assessment of the risks of transmission of microorganisms to humans from consumption of

raw milk was published by VKM in 2006. VKM concluded that: "the risks associated with *E. coli* O157:H7 and other pathogenic *E. coli*, variants of *C. jejuni* and *L. monocytogenes* in raw milk are considered to be high. Efficient distribution to consumers of less virulent microbes by unpasteurised milk may also be of concern because of the need to counteract the ongoing rise in AMR and because of the threat that opportunistic pathogens may pose to immunocompromised individuals" (VKM, 2006).

In their scientific opinion on public health risks associated with raw milk in the EU, experts from EFSA's Panel on Biological Hazards (BIOHAZ) concluded that raw milk can be a source of harmful bacteria – mainly *Campylobacter* spp., *Salmonella* spp., and Shiga toxin-producing *E. coli* (STEC) (EFSA, 2015). These are all bacteria that are well-known carriers and disseminators of AMR.

Mycobacterium bovis is another bacterium that was identified as a main hazard related to raw milk in the EFSA report (2015). *M. bovis* is a zoonotic pathogen and causes tuberculosis in an array of warm-blooded animals, including cattle, llamas, alpacas, dogs, and cats, as well as humans. *M. bovis* has not been detected in the Norwegian cattle population since 1986 (www.vetinst.no). However, the emergence of multi drug-resistant *M. bovis* among humans worldwide may become a challenge, with increasing immigration from countries where the endemic level is considerable. Similar to the LA-MRSA situation in pig production, foreign workers may introduce multi drug-resistant *M. bovis* to the cattle population. From here the bacteria may be re-distributed to the human population through raw milk.

Milk products

Milk is used in an array of dairy products, such as yoghurts, cream, sour cream, kesam, crème fraîche, and soft and hard cheeses. The production processes often involve inhibition of the indigenous milk microbiota by some sort of controlled fermentation. Lactic acid bacteria (LAB) and some species of *Streptococcus* are frequently used for fermentation of milk products. Some of these LAB are commercially available starter cultures that are identified as having a qualified presumption of safety (QPS). Lactobacilli are generally intrinsically resistant to several antimicrobials and this resistance cannot be transferred to other bacteria. However, starter cultures in traditional cheese-making may originate from the bacteria naturally present in raw material. These are often complex microbial consortia consisting of species that reflect local micro-environments, and their content of AMR is unknown.

See also "Preservation and processing techniques potentially affecting resistance"

In Norway, the majority of cheese is made from pasteurised milk, and, according to the regulations, those producers who make cheese from unpasteurised milk are required to take extraordinary hygienic precautions during production (LOVDATA, 2010). During 2012, about

10 % of the Norwegian cheese market consisted of imported cheese (more than 11 000 tonnes), of which 99 % was imported from EU (https://www.slf.dep.no/no/internasjonal-handel/import/tollkvoter/publikasjoner/importvernet-for-ost).

There are no data available on the occurrence of resistant bacteria in Norwegian or imported milk products. However, several international publications report finding antimicrobial resistant bacteria in, for example, cheese, including *S. aureus* (Sasidharan et al., 2011), *Staphylococcus xylosus* (Mikulasova et al., 2014), and *E. faecium* (Delpech et al., 2012).

4.5.4 Fish / fish-products and seafood

Although seafood production in Norway has increased considerably in the last 20-25 years, the use of antimicrobial agents in aquaculture has decreased substantially (Figure 2-2).

The first sites selected for fish farming were often in protected areas, shallow and with little water exchange. Accumulation of sediments from faeces and feed was a common problem. Findings by Husvåg et al. (1991) indicated that introduction of antibacterial agents into such sediments might cause long-term effects regarding the resistance profile of the bacterial flora in the sediments. High levels of AMR among bacteria in marine sediments from aquaculture and non-aquaculture sites suggest that dispersion of the large amounts of antimicrobials used in Chilean salmon aquaculture has created selective pressures in areas of the marine environment, distant from the initial site of use of these agents (Shah et al., 2014). Aquaculture production units today tend to be established in more exposed areas, with stronger currents and better water exchange. This, together with better control of feeding regimes, has led to fewer problems with sediment accumulation under the cages.

Currently, no national or international breakpoint values, relating to MIC (broth dilution) or diffusion zone diameters (disc diffusion) are available for aquatic/fish pathogenic bacteria and therapeutic antimicrobial use. Guidelines for resistance-testing of fish-pathogenic bacteria are directed solely at establishment of standard testing methodologies and do not address interpretative criteria. Standard testing of fish-pathogenic bacterial species at the Norwegian Veterinary Institute is based upon disc diffusion testing. While inhibition zone size cannot be directly related to treatment outcome, zone diameters are monitored and any reduction in 'normal' zone diameter for any particular bacterial species/antimicrobial combination is investigated using molecular methods. The frequency of fish-pathogenic bacteria with reduced susceptibility to antimicrobial agents is low and, over the last 15 years, has been limited to detection of reduced susceptibility to quinolone antimicrobials. Such monitoring has revealed reduced quinolone susceptibility in *Vibrio anguillarum* (Colquhoun et al., 2007), *Yersinia ruckerii* (Shah et al., 2012b), and *Flavobacterium psychrophilum* (Shah et al., 2012a), which, in all cases, have been associated with chromosomal mutations. Resistance development could be associated with either repeated oxolinic acid treatment or introduction of resistant strains to Norway (Nilsen et al., 2014). In addition, reduced

susceptibility to quinolone antimicrobials has been associated with *Aeromonas salmonicida* subsp. *salmonicida* isolated from diseased wild salmon in rivers draining into a fjord on the west coast of Norway. It is suspected, but not confirmed, that this resistance is related to historical treatment of furunculosis in affected fish farms. Intrinsic resistance to antimicrobial agents has been identified within a number of fish-pathogenic bacterial species in Norway. Since intrinsic resistance is not associated with resistance gene(s), HGT to other bacteria does not pose any risk.

4.5.5 Fresh produce (vegetables, fruits, and berries)

The presence of antimicrobial resistant bacteria and/or resistance genes on fresh produce may be due to (i) contamination of the products by soil, manure, or irrigation water, or (ii) prophylactic use of antimicrobials in orchards to combat bacterial plant diseases.

4.5.5.1 Probability of spreading AMR to clinical bacteria

It is well established that bacteria harbouring transmissible antimicrobial genes are common in the environment, even if they have not been exposed to exogenous antimicrobials. It is also clear that antimicrobial applications on plants can favour populations of resistant bacteria present at the time of application. Moreover, antimicrobial resistant bacteria that are competent phyllosphere colonisers can persist in the environment. During application of antimicrobials, the orchard floor will inevitably be contaminated with antimicrobials that could potentially select for pools of AMR genes in soil. However, recent studies do not support this (Popowska et al., 2012; Walsh et al., 2011), perhaps because many antimicrobials are rapidly degraded in the soil.

In some areas in the USA, some acquired streptomycin-resistant isolates of *E. amylovora* have been detected, but no acquired resistance against oxytetracycline. However, such resistance has been found in plant-surface (phylloplane)-associated bacteria (Schnabel and Jones, 1999). Bacteria harbouring transferable genes for gentamicin resistance have been detected in several environments, irrespective of exposure to gentamicin, but it is unclear whether gentamicin resistance will emerge in phytopathogenic bacteria. A relatively rapid emergence of resistance against oxolinic acid has occurred, partly showing cross-resistance to other quinolones, including ciprofloxacin. This has reduced the use of this antimicrobial for controlling plant diseases in many countries.

In a study from the Netherlands, seven vegetable types that had mostly been obtained from supermarkets were studied. In order to determine whether the agricultural environment was the source of ESBL/AmpC-producing *Enterobacteriaceae* on fresh produce, iceberg lettuces were also obtained directly from three farms, together with samples of soil and irrigation water. ESBL/AmpC-producing *Enterobacteriaceae* isolated from vegetables and the

environment were all environmental species, but 3rd-generation cephalosporin-resistant faecal *Enterobacteriaceae* were isolated from 2.7 % of supermarket vegetables, 1.3 % of iceberg lettuces from farms, and 1.1% of agricultural soil. Faecal *Enterobacteriaceae* were all identified as *Citrobacter* and *Enterobacter* species and, with the exception of one *Citrobacter koseri* strain, all had phenotypes indicative of constitutive AmpC production (Blaak et al., 2014).

4.5.5.2 The situation in Norway

The use of antimicrobials in plant agriculture is not allowed in Norway. However, a large number of fungicides are approved for use, including derivatives of azoles. There is presently no evidence for a correlation between the agricultural use of azoles as fungicides and resistance among mycotic human pathogens, but such concerns have been expressed (Hof, 2001).

One product of *Streptomyces* K61 (formerly *S. griseoviridis*) has also been approved for use in Norway as a fungicide. Streptomyces K61 produces an aromatic polyene with antimicrobial activity *in vitro*. The use of *Streptomyces* K61 was negligible in Norway during 2009-2013 (except from 0.1 kg. in 2011), but an application for further approval is under consideration.

Antiviral agents have not yet been used in plants anywhere (Vidaver, 2002).

To the best of our knowledge, susceptibility-testing of bacterial species from fruit and vegetables has never been included as part of a surveillance programme. However, in 1994 an increase in the number of domestic cases of *Shigella sonnei* infection was detected in several European countries, including Norway, Sweden, and the United Kingdom. Epidemiological evidence incriminated imported iceberg lettuce of Spanish origin as the vehicle of transmission. During the laboratory investigations of the outbreak, it was discovered that the imported iceberg lettuce harboured *E. coli* strains showing resistance to several antimicrobial agents, including ampicillin, ciprofloxacin, gentamicin, and trimethoprim-sulphamethoxazole (Kapperud et al., 1995).

4.5.6 Drinking water

Water is one of the most important bacterial habitats, and represents a major vehicle for dissemination of bacteria between different environments (Vaz-Moreira et al., 2014). Water has also been recognized as a significant reservoir of antimicrobial resistant bacteria (Zhang et al., 2009), as an array of transferable AMR genes have been detected in microbial communities both in pristine and contaminated water sources.

Human exposure to antimicrobial resistant bacteria from water may occur via the following routes:

- Consumption of drinking water.
- Introduction to the production value chain through animals' drinking water.
- Use in food processing (see 4.5.7).
- Use as a food ingredient.
- Use as irrigation water.

4.5.6.1 Drinking water for humans

Provision of safe drinking water in Norway is largely based on water sources of high quality, source protection, and water treatment (Nygård, 2008). The Norwegian drinking water regulations require at least two hygienic barriers against all physical, chemical, or microbiological pollution that could possibly affect the potable water supply (LOVDATA, 2001a). With regard to microbiological contamination, drinking water shall not contain coliform bacteria, *E. coli*, intestinal enterococci, or *Clostridium perfringens*, and the total number of bacteria should be low (LOVDATA, 2001a).

In 2011 (the latest available data from Norwegian waterworks registry, FHI 2015), 88 % of the Norwegian population received water from waterworks serving at least 50 persons or 20 households. Surface water is the main water source; 57 % of waterworks used surface water as their source. However, these waterworks supplied 90 % of the population that received water from waterworks above the size limit. This indicates that groundwater waterworks generally serve quite small communities. Deficiencies in microbiological requirements are normally a consequence of inadequate hygienic barriers or internal controls in water work operations.

Pathogenic microorganisms that can be transferred to humans through water include viruses, parasites, and bacteria, and the bacteria may harbour transferable AMR determinants. In a review of waterborne disease burden in developed countries from 2014, the parasites *Cryptosporidium* and *Giardia* were the most common pathogens (Murphy et al., 2014). The bacteria most frequently detected were *Salmonella* spp. *Shigella* spp., *E. coli*, and *Campylobacter* spp. These bacterial species are all known to harbour transferable determinants encoding resistance towards antimicrobials used as therapeutics.

Although municipal drinking water is generally safe, exposure to pathogenic microbes may occur due to specific incidents. These may occur at different levels: 1) from water source and failure in water treatment, 2) due to distribution systems, and 3) extreme weather events. Nygard et al. (2007) studied drinking water distribution systems in Norway and the effects of distribution system failures on gastrointestinal health. A higher risk of gastrointestinal illnesses was found in households exposed to events in the distribution system, such as main breaks or maintenance work, compared with non-exposed households. Ageing drinking water distribution systems may be the cause of leaks in the system, main

breaks, or low-pressure episodes. These types of events can allow pathogens from the surrounding environment to intrude into the drinking water distribution network.

4.5.6.2 Drinking water for animals

Access to drinking water of good quality is important for animal health and welfare, and is also a prerequisite for optimal production. For in-house water supply, most farmers use municipal water supply for their animals. The requirements are that this water shall not contain coliform bacteria, *E. coli*, intestinal enterococci, or *Clostridium perfringens*, and the total number of bacteria should be low (LOVDATA, 2001a). There is, however, a probability that water troughs in animal houses can be contaminated with faecal material from the animals. Hence, drinking water will contribute to the recycling of antimicrobial resistant bacteria if these were originally carried by the animals. When the animals are kept outside, they can use several sources for drinking water. Some animals will have access to surface water, such as lakes, rivers, and streams, whereas others will be provided with drinking water in different types of open containers. Such drinking water sources are vulnerable to contamination from the environment, and may also become contaminated by antimicrobial resistant bacteria of many different origins. Hospital wastewater and livestock manure are considered as major sources of environmental antimicrobial resistant genes (Zhang et al., 2009).

4.5.6.3 Water used as a food ingredient

The same requirements for microbiological quality apply to water to be used as a food ingredient as for drinking water. The fate of bacteria and their genes that are introduced into the production value chain with water will depend on the food processing and storage conditions until consumption.

4.5.7 Food processing

Food processing includes all steps from harvest/slaughtering until the food is eaten by the consumer. Food processing may be very simple (e.g., packaging of fruit and sale at the farm) or include several steps (e.g., production of whole ready-to-eat meals). As many processes are, in principle, similar for a wide range of foods, the impact on bacterial resistance will also be common for several food chains.

Many of the food-production processes include measures that will reduce the load of unwanted microbes on the products, including pathogens and resistant bacteria:

- Elimination or removal from raw materials: e.g., washing steps, removal of outer layers (fur, feathers, shells), heating, drying, decontamination (e.g., radiation), and smoking.

- Prevention of growth: e.g., addition of preservatives, drying, salting, cooling, addition of starter cultures, and packaging.
- Prevention of recontamination: cleaning and disinfection (chemical or heat) of production environments, personal hygiene, process hygiene

Thus, in many cases, food-production processes have a key role in eliminating and reducing contamination, including with AMR bacteria, from the food chain. Therefore, any negative impact of such technologies on resistance has to be weighed against the risk-reduction capacity. There is no direct evidence that food processing contributes to increased resistance to antimicrobials, although laboratory studies indicate that it cannot be excluded that a few processes/technologies might be associated with resistance (SAFEFOOD, 2010; Verraes et al., 2013)

4.5.7.1 Microorganisms on raw materials and products, and in food-processing environments

Whereas raw materials may contain a very wide range of bacterial taxa, the processing and selective pressure on bacteria in products and processing environments often limit the diversity. For example, modified atmosphere packaged ready-to-eat meat with organic acid preservatives mainly harbour LAB and only spore-forming bacteria survive a cleaning process followed by heat disinfection. Environmental bacteria may harbour resistance determinants that can be transferred to pathogens, either in the food or on surfaces in contact with the food. Thus, when considering AMR in food processing, the focus should not only be on pathogens, but also on environmental and commensal bacteria.

The sources for food-associated pathogens may be animals (e.g., *E. coli*, *Campylobacter* spp., *S. aureus*), but also the environment (e.g., *L. monocytogenes, Pseudomonas*), or humans (e.g., *S. aureus*, *E. coli*). Among these, only *L. monocytogenes* (commonly) and *S. aureus* (rarely) are linked to contamination from the processing environment.

4.5.7.2 Contamination of raw materials through the slaughter process

Despite strict hygiene, bacteria, including resistant bacteria from faeces or outer parts of the animal (skin, feathers), may contaminate the raw product during the slaughtering process. There are no indications that processes reducing this contamination, such as rinsing, ultraviolet irradiation, or heat treatment, select for resistant bacteria. Therefore, such technologies should be promoted to reduce the risk of contamination of raw materials. Chemical decontamination of raw materials is prohibited in Norway. Increased occurrence of antimicrobial resistant *E. coli* after such treatments has been reported (Capita and Alonso-Calleja, 2013). The mechanism behind this observation was not studied and further investigation is needed to confirm this.

4.5.7.3 Contamination during food processing

During food processing, raw materials may be contaminated by food operators or from the production environment.

4.5.7.4 Food operators

Personal hygiene for people handling food is regulated by law (Stanga, 2010). For most food-processing plants this means change of clothing, hand washing, and hand disinfection for all personnel entering the food processing/handling areas. It is also common to restrict access of persons, including visitors that have been recently traveling to high risk countries or have been suffering from foodborne infections. Alcohol-based disinfectants are used for hands and the use of ethanol/propanol has never been linked to AMR (see 4.9.2.2).

The impact of food handlers on the probability of spread is difficult to assess, as it would require data on both occurrence of antimicrobial resistant bacteria in food handlers and on the frequency of violation of hygienic rules. The Nordic countries are considered low prevalence countries, but the prevalence is increasing (Brolund, 2014). Several studies show that travelling is an entrance route for antimicrobial resistant bacteria such as ESBL/AmpC-producing *Enterobactericaceae* (Brolund et al., 2014; van der Bij and Pitout, 2012). The prevalence of these bacteria in humans is extremely high (>50%) in some parts of the world, and one study showed that 24 % of travellers to countries outside northern Europe returned to Sweden as new carriers of ESBL/AmpC-producing bacteria. Travels and tourism to higher risk countries are not unusual in Norway, and food handlers from a range of foreign countries are employed in the food processing chain. At the consumer stage, contamination from a known healthy carrier of ESBL/AmpC to other persons in the household has been documented (Lohr et al., 2013), but it has not been shown that food preparation is a contamination route. Similarly, contamination between members of a household has been shown for MRSA, but was associated with sharing soap and towels (Turabelidze et al., 2006). In conclusion, it cannot be ruled out that antimicrobial resistant bacteria may be spread into the food chain through food contaminated by carriers of these bacteria.

4.5.7.5 Water for processing of food

All food production facilities, from slaughterhouses to retail and grocery stores, are required to use water of drinking water quality for processing food. This is regulated in the "Regulation on water supply and drinking water" (LOVDATA, 2001a). Water treatment plants themselves are responsible for delivery of water of acceptable quality, and are obliged to conduct a risk evaluation of their production.

4.5.7.6 Food-processing environment

Sanitation of the food-processing/handling environment is regulated by law (LOVDATA, 2010). Most food production plants clean and disinfect machines and equipment and the processing environment (floors, walls, drains) on a daily basis. The sanitation process removes the majority of bacteria and prevents the introduction of unwanted microorganisms into the production line from one day to the next.

A direct link between resistant bacteria surviving sanitation in the food industry and resulting in infection and failure of antibiotic therapy has not been proven. Also, the efficacy of biocides being equally effective against highly resistant bacteria as sensitive bacteria has been demonstrated (Reichel et al., 2014). However, laboratory studies have shown that the same mechanisms can give both reduced susceptibility to disinfectants and antimicrobials (co-resistance/cross-resistance) (Hegstad et al., 2010). Thus, concerns have been raised about the use of disinfectants and the possible role for emergence of AMR. Table 4-4 gives an overview of commonly active ingredients in washing and disinfection agents used in food-processing environments. Commercial products often contain other components to increase efficacy (e.g. EDTA) or for enhancing technical properties (e.g. foaming agents) (Stanga, 2010). These agents are used broadly in processing of all types of food, including food of animal origin, fish, and fresh produce.

Table 4-4 Commonly used biocides in the food-processing industry and mechanism of action (Stanga, 2010)

Chemistry	Example	Mechanism of action
Cationic tensides	Benzalkonium chloride, didecyldimethylammonium chloride	Absorbs to cell membranes; interacts with proteins and lipids; leakage of cell components
Halogens	Hypochlorite, chlorine dioxide	Oxidizes lipids, proteins, carbohydrates; destroys cell structure and metabolism
Peroxygen	Peracetic acid, peroxide	Oxidizes lipids, proteins, carbohydrates, destroys cell structure and metabolism
Alcohol	Propanol	Denatures and coagulates proteins; destroys cell structures and interacts with metabolism

Cross-resistance and co-resistance between cationic disinfectants and antimicrobials have been a subject of much research, and quaternary ammonium compounds (QACs) are grouped among the high risk biocides of SCENIHR (Capita and Alonso-Calleja, 2013). Most multi-drug efflux pumps that mediate resistance to both QACs and antimicrobials are intrinsic mechanisms. One exception is the plasmid-borne OqxAB gene that encodes resistance against antimicrobials, disinfectants, and detergents (Hansen et al., 2007). There are a number of examples of co-resistance in which QAC efflux pump determinants are located on transferable elements (plasmids and transposons) together with genes encoding AMR (Hegstad et al., 2010), but most studies have been done on clinical isolates. A correlation

between reduced susceptibility to disinfectants and antimicrobials due to co-resistance has been demonstrated in clinical environments (Buffet-Bataillon et al., 2011; Sidhu et al., 2002). So far, no correlation has been found in food environments (Cole et al., 2003; Gantzhom et al., 2014). *Staphylococcus* spp. is a frequent contaminant of food-processing equipment and some strains harbour multi-resistance plasmids (Sidhu et al., 2001). In general, acquired efflux pump mechanisms result in resistance levels far below the concentration used, and the role for survival after practical disinfection is not clear. However, disinfectant agents in high concentrations may leave residues in the environment and will inevitably be diluted to sub-lethal concentrations. This may contribute to a more prolonged effect on bacteria, both at the application site and in the environment, possibly leading to the development of resistance.

4.5.7.7 Preservation and processing techniques potentially affecting resistance

During the production process of certain food products, microorganisms are intentionally added for technical reasons or for claimed positive health effects. Such strains are often ingested on a large scale. They may contribute to the dissemination of AMR if they carry resistance genes or have the potential to be involved in HGT. Starter cultures are microbiological cultures added to foodstuffs to induce the onset of fermentation, e.g., in yoghurt and fermented sausages. Different *Lactobacillus* species are mostly used for such purposes.

Probiotics are live microorganisms that are added to foodstuffs for a claimed positive health effect for the consumer. *Lactobacillus* and *Bifidobacterium* are most frequently used, but yeasts like *Saccharomyces boulardii* can also be employed. Probiotic microbes are becoming more widely used globally as food supplements, and they may also be used in food-producing animals and aquaculture production. Starter cultures, probiotics, and biopreserving microorganisms will often be consumed in large amounts, and end up in – and partly colonise - the human gastrointestinal tract where resistance genes, if present, can be transferred. Such transfer will usually occur by conjugation to other members of the intestinal microbiota. Historically, resistance genes were not part of the standard screening assays for starter cultures and probiotics. It is important that bacterial cultures intended for such use are characterized at the strain level, not only for the absence of resistance genes but also for the potential for both acquisition and dissemination of such genes via HGT mechanisms (Wang et al., 2012).

4.5.8 Sewage and manure

Low concentrations of antibacterial compounds may be excreted in urine and faeces from humans and animals receiving antimicrobials for prophylactic or therapeutic purposes. Consequently, many antimicrobial agents and their metabolites will eventually end up in

wastewater, sludge, or animal waste, from where they can be introduced to new recipients and environments (Kummerer et al., 2000). In particular, waste and run-off from hospitals and the pharmaceutical industry may represent sources of antibacterial agents and potentially resistant bacteria.

Sewage sludge and animal waste are used as fertilizing agents in the production of food crops. Recycling of nutrients is recommended for environmental and agricultural reasons. However, this practice may also introduce low doses of antimicrobials and resistant bacteria into human food chains. In Norway, sewage sludge cannot legally be applied to soil used for growing fresh produce (vegetables, berries, fruits), on pasture, or in plant nurseries. After sludge amendment, at least three years must elapse before fresh produce can be grown. Sewage sludge has to be mixed into the soil immediately, and, at the latest, 18 hours after application. Sludge has to meet certain quality criteria (maximum limits of heavy metals and thermotolerant coliform bacteria, no *Salmonella* spp. or parasite eggs).

In 2009 VKM published an assessment of the risk of using sewage sludge as fertilizer and soil conditioner in agricultural lands and park areas, as well as sludge mixed with soil sold to private households. The report concluded that it was unlikely that AMR would be promoted in the sewage treatment plant (STP) water, in the sludge, or in the soil following application of sewage sludge as fertilizer. Development of resistance to the fluoroquinolone ciprofloxacin in soil was mentioned as a potential exception (VKM, 2009).

4.6 Summary of hazard characterization

Transmission of AMR from livestock to humans through food varies according to the bacteria and type of resistance.

The most important hazards related to AMR in the Norwegian food chains are: LA-MRSA from pigs, ESBL/AmpC-producing bacteria belonging to *Enterobacteriaceae* in poultry, vancomycin-resistant enterococci in poultry, quinolone-resistant *Campylobacter* and *E. coli* isolated from poultry.

The prevalence of antimicrobial resistant bacteria isolated from fish and fish products is low. Our knowledge regarding antimicrobial resistant bacteria among imported aquaculture products is limited.

The data regarding antimicrobial resistant bacteria from Norwegian cattle indicate low occurrence of resistance among indicator bacteria. Resistance to fluoroquinolones among *E. coli* has never been detected. ESBL/AmpC-screening by using a selective method has not been carried out on samples from cattle. The regulations require that almost all milk for consumption is pasteurised at the dairy.

No data are available on the occurrence of resistant bacteria in Norwegian or imported milk products. However, several international publications report finding antimicrobial resistant bacteria in, for example, cheese, including *S. aureus*, *S. xylosus*, and *E. faecium*.

Use of antimicrobials in plants has never been approved in Norway, and susceptibility-testing of bacterial species from fresh produce has not been included as part of a surveillance programme in Norway. There is a lack of data regarding antimicrobial resistant bacteria from imported vegetables and fruits in Norway.

Provision of safe drinking water in Norway is based on water sources of high quality, source protection, and water treatment. The Norwegian drinking water regulations require at least two hygienic barriers against all physical, chemical, or microbiological pollution that could possibly affect the potable water supply. With regard to microbiological contamination, drinking water shall not contain coliform bacteria, *E. coli*, intestinal enterococci, or *C. perfringens*, and the total number of bacteria should be low. Antimicrobial susceptibility data from bacteria isolated from drinking water in Norway are lacking.

Biocides, such as QACs, used in food processing may select for tolerant bacteria with resistance to antimicrobials. Although some studies regarding biocide resistance in bacteria originating from food and co-selection have been conducted, there is a lack of monitoring and knowledge about clinical impact in humans.

5 Exposure assessment: human exposure from food chains in Norway

Antimicrobial agents are used in different agricultural sectors and at different stages of production, including animal feed, food-producing animals, crop production, and/or during food processing (CAC, 2012).

Following antimicrobial use, selection of resistant microorganisms and determinants may occur. These could then be disseminated between the different stages in the food chains. Considerations related to exposure assessment are illustrated in Figure 5-1.

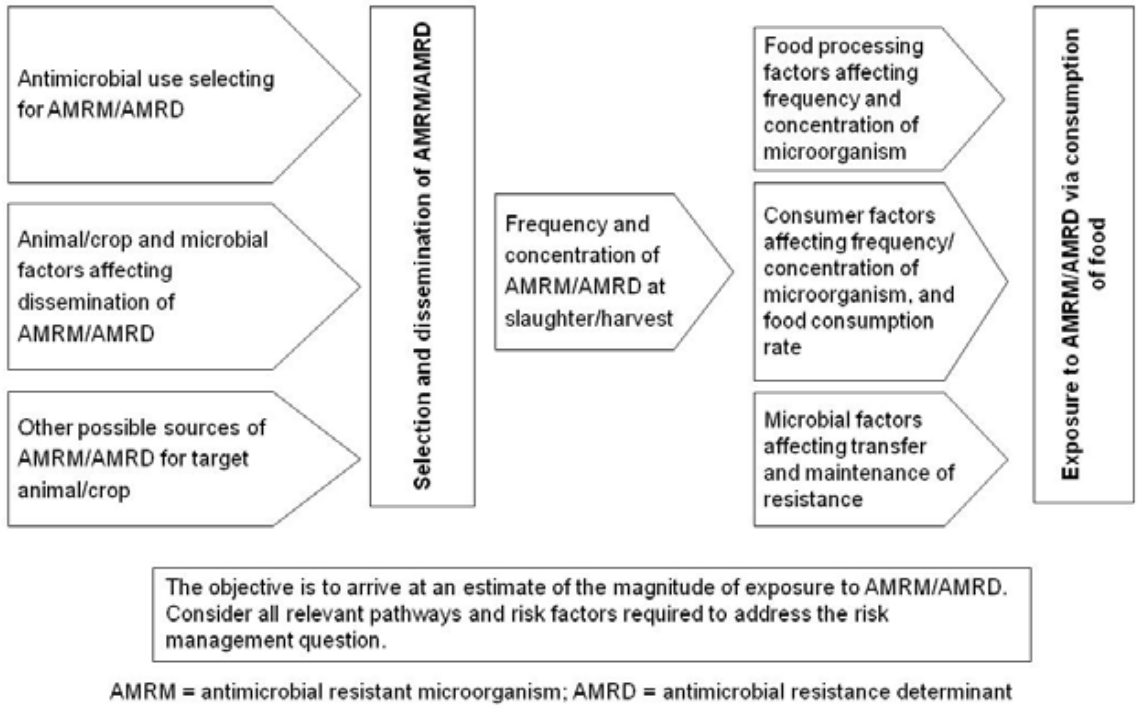


Figure 5-1. Considerations for Exposure Assessment in a Foodborne AMR Risk Assessment - the Exposure Pathway (CAC, 2012).

When considering exposure of humans to AMR, VKM assumes that the probability of occurrence of AMR in the respective food chains reflects the probability of human exposure to that resistance via the same food chains. For a comprehensive risk assessment, these data should then be related to clinical data from humans, including: a) burden of disease due to exposure, b) number of treatments with antimicrobials, and c) number of ”failures”,

versus ”cures”. However, as such data are not available, only the probability for transmission of AMR to humans has been evaluated and not the consequences of transmission of resistant bacteria or AMR determinants. VKM is aware that there are many important data gaps and that these make the assessment of exposure of humans to AMR complicated.

Foodborne pathogens will almost always account for only a small percentage of the total food microbiota. How often commensals and non-pathogenic environmental bacteria may contaminate food is largely unknown, but as such microbes will normally outnumber pathogens, they will be present far more frequently and at higher numbers. While the occurrence of antimicrobial resistant pathogens is alarming, the magnitude of the AMR gene pool in foodborne commensals is yet to be revealed. However, it is probably safe to assume that such organisms will always be present in food to some extent, unless adequate risk-reduction management practices have been adopted.

In accordance to the proposal by Codex (CAC, 2012), the rankings “Negligible,” “Low,” “Medium,” “High” and “Not assessable” are used for qualitative determination of the probability of human exposure to a given AMR in a given food or feed commodity, animal species, or plant. The different ranking categories are defined below (CAC, 2012):

- Negligible – The probability of exposure of susceptible people is extremely low;
- Low (Unlikely) – The probability of exposure of susceptible people is low, but possible;
- Medium (Likely/Probable) – The probability of exposure of susceptible people is likely;
- High (Almost Certain) – The probability of exposure of susceptible people is certain or very high;
- Not assessable – The probability of exposure of susceptible people cannot be assessed.

In this assessment all ranking categories other than “**negligible**” have been combined in the category “**non-negligible**” (see 6.4).

5.1 Food consumption in Norway

Table 5-1 shows developments in meat consumption over the last decade in Norway. Consumption is calculated from wholesale figures showing annual consumption in kg per capita. The numbers for chicken and turkey are not separated, but the stakeholders’ calculations show a 90/10 distribution, where chicken represents 90 % while turkey is 10 % of the consumption of poultry meat. Ducks and geese are not included, and their production and consumption is very low in comparison.

Table 5-1. Consumption of meat in kg per capita, Norway 2005-2015 (Norsk institutt for landbruksforskning, NILF)

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014*	2015*
Beef	19.4	19.2	20.0	20.1	18.5	18.3	18.6	19.0	18.6	17.9	18.1
Veal	0.4	0.5	0.4	0.4	0.3	0.3	0.4	0.4	0.5	0.4	0.4
Sheep/lamb	6.1	5.7	5.6	6.0	5.2	5.5	5.1	5.2	5.2	5.0	5.2
Pork	24.8	24.8	26.9	26.0	25.4	25.6	26.3	25.6	24.9	25.6	25.7
Poultry	12.6	13.5	14.9	16.6	17.7	16.9	17.5	18.4	20.6	19.6	18.4

*Consumption for 2014 and 2015 are estimates only

As shown in the table, the consumption data are relatively stable except for an increase in the consumption of poultry. This growth in consumption had a slight dip in the course of 2014 and 2015.

At the same time, there is a trend for increasing consumption of fresh produce in Norway (Figure 5-2). Such foods when contaminated by animal waste or contaminated water may also constitute a transmission route for antimicrobial resistant bacteria.

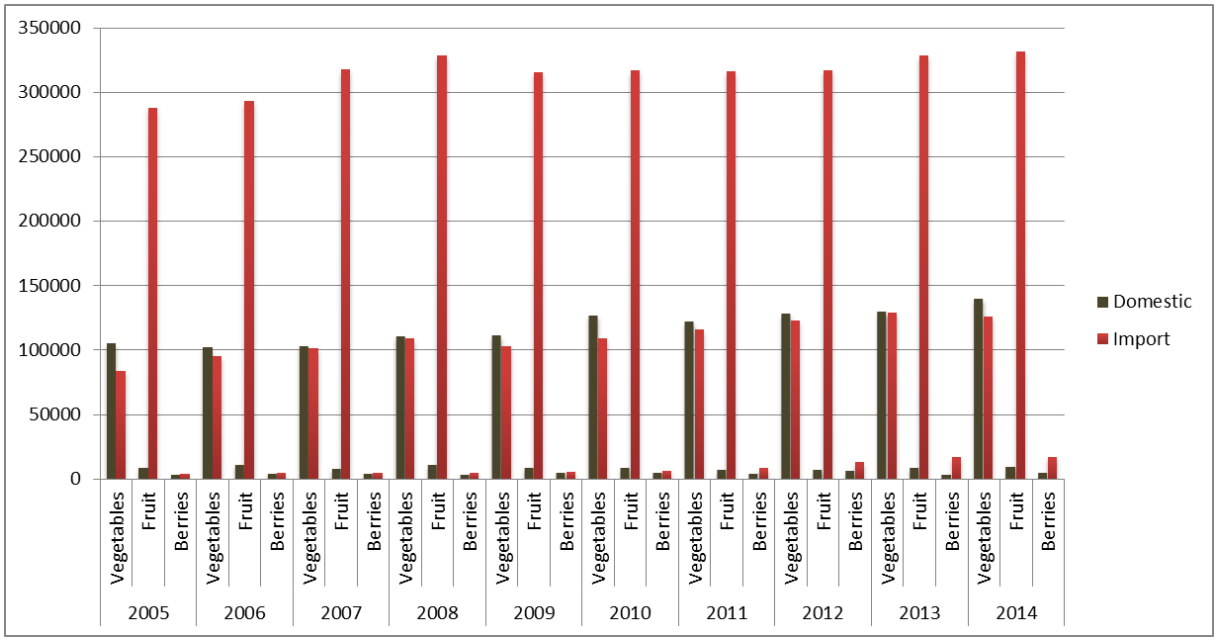


Figure 5-2. Commercial sale of fresh fruit, berries and vegetables in tonnes (Norwegian and imports) at wholesale level, in Norway 2005-2014 (Opplysningskontoret for frukt www.frukt.no).

5.1.1 Exposure via pigs and pork products

5.1.1.1 MRSA

A limited occurrence of LA-MRSA has been identified in Norwegian pig farms during recent years (see 4.3.2). As genotyping studies have demonstrated the occurrence of the same LA-MRSA strain in humans and animals, transmission between animals and humans has probably occurred (Angen et al., 2015). People with direct contact with animals, such as farmers, farmworkers, and veterinarians, have been exposed to, and found to carry, LA-MRSA. Different MRSA spa-types (see glossary for more information) have been detected from pigs in Norway, including: t008, t011, and t034. MRSA t008 was found on a single pig farm in 2008 and transmission from humans on the farm was suggested (Sunde et al., 2011a). MRSA t011 and t034 are associated with clonal complex (CC) 398 and are common LA-MRSA variants among pigs (and other livestock animals) on a global basis. The recent LA-MRSA cases in Norway involved t034 and, to a lesser extent, t011 (Angen et al., 2015). MRSA of spa-type t127 has also been detected in pigs in Norway (Angen et al., 2015)

The most commonly found MRSA variants among humans in Norway are: t002, t019, and t008 (NRLMRSA, 2014). The livestock-associated spa-types, such as t034 and t011, are not common among humans in Norway. The increase in occurrence of t011 and t034 in 2013 and 2014 can probably be attributed to targeted screening of persons who are exposed to MRSA-positive pigs in the specific LA-MRSA cases.

Transmission of ESBL/AmpC-producing bacteria from food-producing animals to farmers and households has not been studied in Norway. However, a Dutch research group demonstrated that daily exposure to pigs carrying ESBL/AmpC-producing *Enterobacteriaceae* is associated with ESBL/AmpC carriage in humans (Dohmen et al., 2015).

Direct transmission from live, LA-MRSA carrying animals to humans is likely. However, the goal is to prevent the establishment of a LA-MRSA reservoir in the pig population.

Although environmental contamination with MRSA plays a role in the acquisition of MRSA in farmers and their household members, transmission of MRSA to humans due to consumption of food is considered very low. However, the dynamic of that transmission may be changed due to evolutionary processes that enable the strain to adapt to a human lifestyle. The transfer of MRSA via raw food due to insufficient kitchen hygiene cannot be discounted.

5.1.1.2 Other resistant bacteria

The occurrence of resistant variants of the indicator bacterium *E. coli* in pigs and pork products of Norwegian origin is low to moderate. Exposure to VRE, QREC, and ESBL/AmpC is low, as these bacteria occur at low or negligible rates in Norwegian pig production. As

Norway is almost free of *Salmonella* spp. in livestock, the probability of exposure to antimicrobial resistant *Salmonella* is negligible.

Campylobacter coli was detected in the gastrointestinal tract of all pigs examined, and the high contamination of tonsils (66.7 %) and intestinal tract (100 %) might represent an occupational health hazard (Nesbakken et al., 2003). Data regarding susceptibility to antimicrobial agents of these isolates are lacking.

Thus, the probability of exposure of humans to such bacteria in Norway is considered to be low.

5.1.2 Exposure via poultry, poultry products, and eggs

Poultry meat available at retail in Norway is, with very few exceptions, produced in Norway.

5.1.2.1 ESBL/AmpC

Exposure to resistant bacteria via poultry and poultry products of Norwegian origin is possible, as both broilers and poultry products can contain cephalosporin-resistant *E. coli*. However, the prevalence of cephalosporin-resistant isolates in poultry products compared with other *E. coli* isolates (cephalosporin-susceptible) is generally low.

Cephalosporin resistance in *E. coli* from Norwegian broiler production can mainly be explained by the presence of the bla_{CMY-2} gene (Mo et al., 2014), which belongs to class C Ambler classification of β-lactamases of Gram-negative bacteria.

Approximately 2 % of *E. coli* isolates of human origin that cause UTI and 5 % of those causing BSI are resistant to 3rd-generation cephalosporins (NORM/NORM-VET, 2013). Genes of the bla_{CTX-M} group play a major role in conferring cephalosporin resistance, whereas plasmid-mediated AmpC genes, like the bla_{CMY-2} gene, are less prevalent in humans in Norway. However, a recent study has investigated human bla_{CMY-2} carrying *E. coli* from UTI and BSI in order to investigate a possible overlap between poultry and human reservoirs (Berg et al., 2015). The study concluded that highly similar *E. coli* strains, belonging to sequence type (ST) 38 and carrying bla_{CMY-2}, occurred in both poultry and humans with infections. In addition, closely related plasmids carrying the bla_{CMY-2} gene were found in *E. coli* variants other than ST38 that were responsible for UTI and BSI in humans. The prevalence of human *E. coli* with characteristics resembling those from a poultry reservoir is probably very low, indicating that transmission from a poultry reservoir to humans is rare.

Acquired metallo-beta-lactamase-producing bacteria and corresponding genes (class B) have not been reported from poultry in Norway to date.

5.1.2.2 VRE

In 2011, NORM-VET used a selective method that demonstrated that 16 % of poultry (or chicken) flocks were positive for VRE. This means that a limited reservoir of VRE exists in the animal population (see 4.3.1).

A study of a European collection of van-A type VRE from humans, foods, and animals strongly suggested that vertical transmission between different reservoirs occurs infrequently (Biavasco et al., 2007). However, VRE of animal origin (*E. faecium* from poultry and pigs) have been shown to be able to colonize the intestinal tracts of humans transiently after ingestion. This indicates that animal VRE can survive passage through the human gastric tract, can multiply in the intestines, and can be isolated in the faeces for up to 14 days after ingestion (Sørensen et al., 2001). Humans (farmers, slaughterhouse workers, and area residents) have also been shown to become temporary carriers of VRE after contact with VRE-carrying animals such as turkeys (van den Bogaard et al., 1997).

In Norway, follow-up studies of VRE after the use of avoparcin as a feed additive was banned from poultry farms in 1995, documented a continuing high prevalence of vanA-type VRE several years after the ban was implemented (Borgen et al., 2000; Sorum et al., 2004), both among humans and in the poultry environment. By using selective methods, VRE was isolated from 6.7 % of broiler flocks. All isolates were identified as *E. faecium* carrying the *vanA* gene (NORM/NORM-VET, 2014).

During transient colonization of the human gastrointestinal tract, animal-VRE may transfer the genes encoding vancomycin resistance to the resident microbiota. Transfer of *vanA* from *E. faecium* isolates of animal origin to *E. faecium* isolates of human origin in the intestine of gnotobiotic mice, and also in healthy human volunteers has been reported (Dahl et al., 2007).

Using surveillance data on *E. faecium* from broilers in Norway, a statistical association was detected between resistance against narasin and bacitracin, as well as between resistance against narasin and vancomycin (VKM, 2015b). We are not aware of studies that have analysed enterococci isolated from humans for resistance against anticoccidal agents used as feed additive in poultry in Norway. Such studies may reveal the possibility of coccidiostatic-resistant enterococci being able to colonize humans.

The probability of exposure to VRE/*VanA* via food is considered low.

5.1.2.3 QREC

According to the NORM/NORMVET(2013 and (2014), the prevalence of QREC in both broiler and turkey production in Norway is high (89.5 % and 70.7 % of broiler caecal samples and broiler meat samples, respectively).

Similar trends have been reported from other countries in Europe (EFSA/ECDC, 2011; EFSA/ECDC, 2012; EFSA/ECDC, 2013). Therefore, human exposure to QREC via poultry products is possible. Resistance to quinolones in bacteria is usually caused by mutations in the quinolone-resistance determining region (QRDR) involving the genes *gyrA, gyrB, ParC,* and *ParE*. In addition, PMQR genes (*qnrA, qnrB, qnrC, qnrD, qnrS, qepA, aac(6')-1b-cr, oqxAB*) are responsible for low-level resistance to quinolones. Among the current isolates, a few had MIC profiles corresponding to the possible presence of PMQR genes, indicating that the resistance is probably mediated by mutations in the QRDR for the vast majority of isolates. This is supported by preliminary results from a pilot study performed by the industry that found resistance to be caused by mutations in the *gyrA* gene (NORM/NORM-VET, 2014).

It is recommended that poultry meat should be well heat-treated before consumption, and the probability of exposure through food intake is therefore negligible. However, exposure to resistant bacteria is possible during meat preparation, but can be minimized if proper kitchen hygiene is practiced to avoid cross-contamination and re-contamination. Currently there are no data that support the QREC reservoir in poultry having affected the QREC rates among human clinical *E. coli* isolates in Norway. An on-going research project is investigating whether transmission of QREC from poultry to humans has occurred.

5.1.2.4 MRSA

LA-MRSA has never been reported from poultry in Norway. Although there have been no systematic screenings with selective methods for detection of LA-MRSA from poultry in Norway, LA-MRSA has not been reported from the diagnostic veterinary laboratories. Studies from other countries have demonstrated that poultry can be colonized with LA-MRSA (Nemeghaire et al., 2013). At present the probability of exposure to LA-MRSA via Norwegian poultry products is negligible.

Salmonella:

Norway is almost free of *Salmonella* spp. in livestock; the probability of exposure to *Salmonella* from poultry products is therefore negligible.

Campylobacter:

The occurrence of *Campylobacter* spp. in Norwegian broiler production is low (4-5 %). In addition, meat from positive flocks is heat-treated or stored frozen for three weeks in order

to inactivate the bacteria before distribution to retailers. The resistance rates, including resistance to quinolones, in *Campylobacter* spp. from Norwegian broilers are low; therefore the probability of exposure to antimicrobial resistant *Campylobacter* from poultry in Norway is low.

5.1.3 Exposure via cattle

People may be exposed to antimicrobial resistant bacteria via direct contact with animals or their faeces, from the cattle food chain through consumption of meat and meat products, milk and milk products, and other products contaminated by manure from cattle (fresh produce, water).

5.1.3.1 Indicator bacteria in live animals and meat

According to the NORM-VET programme, faecal indicator bacteria from meat and faeces (*E. coli, E. faecium, and E. faecalis*) have been shown to express resistance towards antimicrobials, such as streptomycin, erythromycin, and tetracycline. The percentage of resistance phenotypes are <25 %, mostly <15 %. Although individual case studies have been reported, the extent to which the genetic determinants encoding these resistance phenotypes are similar or identical to the determinants that are responsible for the same resistance phenotypes in bacteria from humans is not known. Meat is mostly consumed heat-treated, and vegetative bacteria will not survive the heating process. Whether, and to which extent, resistance genes from dead bacteria in the meat can be taken up and integrated into the genome of other resident gut bacteria is unknown.

5.1.3.2 Exposure via milk

Milk from animals with clinical mastitis is not supposed to reach the production value chain. However, in subclinical mastitis the udder and the milk appear normal, but microorganisms can nevertheless be cultured from the milk. Milk from subclinical mastitis may thus reach the production value chain, but the vegetative bacteria will be eliminated by pasteurisation.

5.1.3.3 Zoonotic pathogens

The foodborne pathogens *Salmonella* spp. *Campylobacter* spp., and zoonotic and pathogenic *E. coli* have a very low prevalence in the Norwegian cattle population. The probable exposure to antimicrobial resistant variants of these pathogens is considered low/negligible.

Although the probability of human exposure to antimicrobial resistant bacteria directly from the cattle food chain is considered low, cattle can contribute to increased levels of resistance through the spread of resistant bacteria via manure. Indirect exposure resulting from

contamination of fresh produce is discussed in section 5.1.5, but it has also been shown that manure fertilization of soil, using manure from cattle that had not received antimicrobial treatment, resulted in a bloom in the resident antimicrobial resistant bacteria (Udikovic-Kolic et al., 2014). The factors driving proliferation of these resident soil bacteria are not known.

5.1.4 Exposure via fish, fish products, and other seafood

Due to limited use of antimicrobials and low levels of resistant bacteria, the probability of human exposure through this food chain is considered negligible. Bacteria from fish and bacteria adapted to humans generally have different optimal temperatures for growth, and psychrophilic marine bacteria seldom colonize humans.

The occurrence of AMR in *Listeria* spp., which may be found in products like smoked fish due to introduction during food processing, is low in Norway. Our knowledge about AMR profiles in imported fish, fish products, and other seafoods is limited.

5.1.5 Exposure via fresh produce

The greatest probability for transmitting antimicrobial resistant bacteria or AMR genes to humans via fresh produce is by consumption of food items that have been accidentally contaminated with antimicrobial resistant microorganisms. The use of antimicrobials in animal husbandry has promoted the development and abundance of AMR in farm environments. Accordingly, manure has become a major reservoir of resistant bacteria and antimicrobial compounds, and its application to agricultural soils is assumed to increase AMR genes and selection of resistant bacterial populations in soil significantly (Heuer et al., 2011). The reservoir of AMR may contain a broad range of different genera and species, of which some have their primary reservoir in the environment, others in animals and/or humans, originating mainly from human or animal faecal material. A significant number of these shares the ability to thrive in different conditions and across hosts, including humans, where encounter and exchange with other organisms, including pathogens, is easy.

As the possible contamination reservoirs for fresh produce will, in general, be the same as for animals, the contamination flora on vegetables and animals respectively will be very similar.

Contamination with bacteria (or their genes) can occur at any point in the farm-to-table continuum. It can occur directly by contact with faecal material or sewage discharges, or indirectly via contaminated environments, irrigation water, or any production or processing equipment. It may also be caused by cross-contamination by resistant bacteria or genetic determinants of AMR originating from other food products during handling of the food by the producer, supplier, or consumer. The use of antimicrobials in plant agriculture is not allowed in Norway. However, fresh produce is susceptible to contamination by resistant bacteria or

genes in the form of commensals or foodborne pathogens, either from the production environment (i.e. soil, irrigation water, manure, wild animals) or as a result of human handling from farm-to-fork. The probability of exposure to such organisms is probably increasing as, overall, the demand for raw or minimally processed food is increasing. Many products are consumed with only minimal or no prior processing steps that could reduce or eradicate microbial contamination.

To our knowledge, systematic surveillance programmes for bacterial contamination of fresh produce in Norway have never been established. The contamination rate with pathogenic bacteria of such products seems, however, to be rather rare. During the period 2010 to 2014, a total of 260 foodborne outbreaks with identified sources were registered in VESUV (FHI, 2015). Of these outbreaks only 16 (6 %) could be traced to different sorts of fresh produce, the majority of which was probably imported. It is also highly likely that the bacteria found in imported fresh produce, in particular from countries with a high prevalence of AMR, are also present in these products. The prevalence of resistant bacteria is considered to be higher in the imported fresh produce than in domestic products.

5.1.6 Exposure via water

Minimal data exist on the prevalence and concentrations of enteric pathogens in surface water, groundwater, or groundwater under the direct influence of surface water. Data are sparse on the concentrations of microbial pathogens in groundwater under non-outbreak conditions. Exposure is therefore difficult to assess.

5.1.7 Exposure via food processing

Food processing will, in general, reduce the levels of resistant bacteria on products. The impact of disinfecting agents on reducing AMR from food chains is most likely much greater than the potential for spread of resistant clones due to co/cross-resistance. Recontamination of products from food handlers may occur, but exposure due to this route is unknown. The contamination of food from bacteria/resistant bacteria with the ability to develop biofilms in food-processing plants cannot be dismissed.

5.1.8 Exposure via sewage

It is unlikely that antibacterial resistance is promoted in STP water, in the sludge, or in soil following application of sewage sludge as fertilizer. An exception may be the potential development of resistance to the fluoroquinolone ciprofloxacin in soil due to the persistence and limited mobility of these substances into the subsoil (VKM, 2009).

Various sludge treatment methods are used prior to the application of sludge in agriculture in order to remove pathogenic microorganisms. Such treatments may also reduce the presence of pharmacologically active substances. It should be noted that naturally occurring antibacterial drugs (antimicrobials) often degrade more readily in the environment than synthetic antibacterial drugs (Jorgensen and Halling-Sorensen, 2000; VKM, 2009).

5.2 The importance of imported animals and food

Due to absence of surveillance programmes, our knowledge about AMR profiles in bacteria isolated from imported food in Norway is limited. There is also no information regarding AMR in illegal imports.

5.3 Summary of exposure

Based on data collected from Norway, the hazard regarding exposure to antimicrobial resistant bacteria via food chains involving cattle, milk, milk products, fish, fish products and seafood, fresh produce (vegetables, fruits, and berries), water, and food-processing to humans is low. However, data for many of these foods and food products, in particular data on occurrence of AMR in imported fresh produce, which represent an important part of consumption, are lacking.

The possibility of transmission of LA-MRSA via food has been the subject of increased attention both in Norway and internationally. Thus far, data from Norway do not implicate MRSA from pigs as a foodborne pathogen. Poultry and poultry products may be the most important reservoir of ESBL/AmpC-producing bacteria and QREC and their resistance determinants, compared with other products of animal origin.

The present extent of transfer of resistant bacteria or genetic determinants of AMR through food is difficult to determine, and the role of food in the transfer of resistance genes has not been fully explored to date.

6 Probability characterisation

Risk characterisation is defined as the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence multiplied by the severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment. Due to data constraints, AMR in Norwegian food chains has been assessed only in terms of probabilities of exposure to humans. In this assessment we discuss the probability of transmission of antimicrobial resistant bacteria or AMR determinants in a qualitative manner, and do not address the risk due to the consequences (disease, hospitalization, death, etc.), as information enabling such an assessment of consequences is lacking (see Uncertainties).

There are classes of antimicrobial agents that are listed both as critically important for human health by WHO and critically important for animal health by OIE. This overlap highlights the need for AMR surveillance and appropriate management measures in order to mitigate resistance dissemination and maintain the efficacy of the drugs (FAO/WHO, 2008). Development of resistance to all classes/types of antimicrobials (both narrow-spectrum and broad-spectrum) is undesirable. However, in this assessment we focus on the most important resistances in bacteria (in particular resistance to 3rd and 4th generation cephalosporins, vancomycin, methicillin, and quinolones). This priority is mainly based on their emergence (see section 4.3), their zoonotic potential, their pathogenicity, and the relative lack of antimicrobial alternatives to treat infections, due to resistance in some strains or clones.

Foodborne pathogens account for only a small percentage of the bacteria present in food and the number of antimicrobial resistant pathogens is even smaller. However, foodborne commensals and environmental bacteria may constitute large pools of AMR genes that can be transferred to human resident and pathogenic bacteria by natural HGT mechanisms (Wang et al., 2006; Wang and Schaffner, 2011). These bacteria, although some may only be transient and do not colonize the intestinal tract, reside long enough to interact with the host microbiota and may possibly acquire or release genes (Marshall et al., 2009). Commensal and environmental bacteria can also act as opportunistic pathogens in susceptible hosts, and probably play a key role in the evolution and dissemination of AMR. Accordingly, a shift in focus from the relatively low number of specific foodborne pathogens (i.e., direct hazards) to the vast number of food bacteria present in food and their genes (i.e., indirect hazards) is necessary in order to assess the probability of AMR in the food chains. For example, the broad term “antimicrobial resistant microorganisms” when discussing the risks associated with the food chains has been used in the last edition of Codex Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance, instead of antimicrobial resistant pathogenic bacteria

(CAC, 2012). This reflects the recognition of the significance of commensal bacteria in the AMR arena (Wang and Schaffner, 2011).

When food items that contain pools of resistant bacteria are consumed without further processing, or only exposed to minimal processing, large numbers of these bacteria are directly transferred to humans. As a consequence, transfer of resistance genes between bacteria may occur after ingestion by humans (Verraes et al., 2013). Food-processing technologies or preservation treatments, like cooling, freezing, acidification, UV-irradiation, or use of salt, aimed at control of growth and a subsequent decrease in microbial load, may also result in stressed or sub-lethally injured cells that can become donors of AMR (McMahon et al., 2007; Wesche et al., 2009). Other food-processing methods are applied in order to kill bacterial cells. Dead cells cannot pass AMR genes to other bacteria by conjugation or transduction, but they may release bacterial DNA, including AMR genes, into the environment. Such genes can, theoretically, be transferred to other cells by transformation. Transformation is, however, subject to a large number of requirements. According to current knowledge, food-processing methods aimed at killing bacteria result in the lowest probability of AMR transfer (Verraes et al., 2013).

6.1 Most important factors inducing AMR

6.1.1 Use of antimicrobial agents for treatment or prophylaxis

Use of antimicrobial agents is probably the most important factor that contributes to development of resistance. Bacterial resistance has evolved with the increased number, volume, and diversity of antimicrobial applications. As new drugs are introduced clinically, resistant strains are identified relatively soon afterwards. The majority of these resistant bacteria are not obligate pathogens, but are part of the indigenous microflora (Barbosa and Levy, 2000). In addition, some active antimicrobials, e.g., quinolones, accumulate and are stable in the environment where their selective effects are perpetuated. Post-treament excretion (antimicrobials excreted in human and animal faeces) may contribute to the level of resistance found in community and environmental pathogens and commensals. This phenomenon, which is known as the eco-shadow concept, is considered a new way of following environmental impacts of antimicrobials (Midtvedt, 2001).

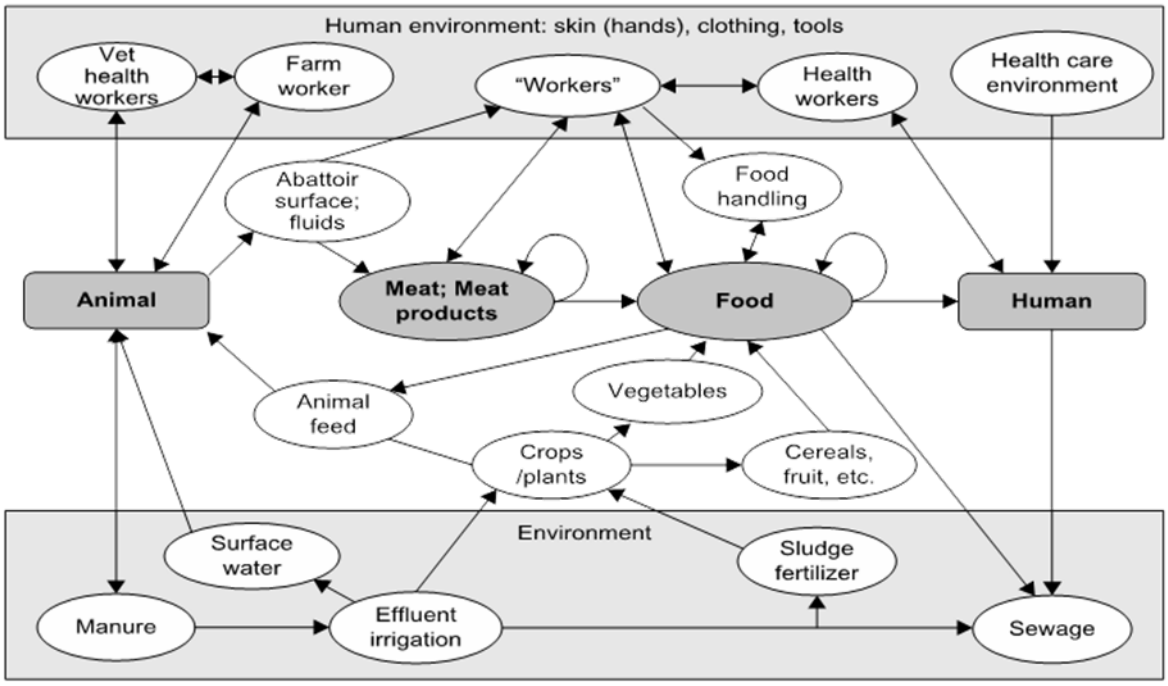
6.1.2 Animal feed

Antimicrobial growth promoters and other substances with an antimicrobial effect are likely to select for resistant bacteria. In the EU, the use of AGP has been prohibited since 1998 (Norway 1995), however, narasin, which is still used as coccidiostatic, has a known antibacterial effect. The risks associated with the use of narasin have been assessed in a separate report from (VKM, 2015b). Feed additive agents, like Cu and Zn, may also

contribute to development of AMR in bacteria (Yazdankhah et al., 2014). For example, a link has been observed between methicillin resistance in staphylococci and use of Zn, as well as a link between vancomycin resistance in *Enterococcus* spp. and use of Cu in animal feed.

6.2 Possible exposure pathways from the food chain

The relationship between AMR in food chains and in humans is complex, as illustrated in Figure 6-1.



Figur 6-1 Possible exposure pathways between animals and humans (Claycamp, 2015).

For both microbial risks and AMR risks, the exposure pathway from farm-to-fork might be highly complex due to numerous indirect pathways. Additionally, colonization with a resistant commensal strain complicates exposure scenarios, with possible time lags between release at the farm animal source and expression of resistance when human patients are treated with antimicrobials.

Several factors and pathways may contribute to induction and spread of AMR. The contribution of each factor is, in addition to a number of other variables, also dependent on the policies for antimicrobial use in different countries. Some of the most important factors and possible exposure pathways are presented below:

6.2.1 Abattoirs

Intestinal contents, faeces, mucosa, and skin from slaughtered animals contain huge numbers of bacteria, some of which may be carriers of AMR. Contamination of carcasses from these sources is a major concern for food safety.

6.2.2 Open population

The probability of transmission of resistant bacteria from one country to another grows as the "global village" shrinks. Strains can be imported into a country and disseminated before their presence is recognized, and countries vary in their capacity to detect and combat resistant bacteria once introduced. For these reasons, the emergence of a resistant bacterial strain in one location rapidly becomes a global problem (Okeke and Edelman, 2001).

6.2.3 Import of animals and foodstuffs

Import of live animals and food products may introduce antimicrobial resistant bacteria, including bacteria with new resistance patterns.

Antimicrobial resistant bacteria, such as *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Enterococcus* spp., and LA-MRSA from animals, may colonize and infect humans who are in daily contact with animals (occupational exposure).

Occupational exposure: People can carry antimicrobial resistant bacteria without any signs of infection. Farmers, farm workers, veterinarians, and others who are in contact with live animals can transfer these bacteria to animals, and vice versa. Similarly, workers in food-processing operations at all levels through the food chains and who carry antimicrobial resistant bacteria may also be a source of contamination.

6.3 Qualitative description of probability

The ranks "Negligible," "Low," "Medium," "High" and "Not Assessable" are defined under Section 5. As emphasised above, the selection pressure from usage of antimicrobial agents is considered the major driving force for development and persistence of AMR. As the usage of antimicrobial agents in Norwegian livestock production is low compared with that in most European countries, the corresponding selection pressure is also lower.

At an international level, it appears that the highest prevalence of resistant bacteria is found in poultry, followed by pigs, cattle, and, finally, sheep. In general, the cattle food chain is associated with fewer antimicrobial resistant bacteria than animals subjected to more intensive production, such as pigs and poultry. This view is supported by a semi-quantitative

risk assessment model developed by Presi et al. (2009), which indicated that antimicrobial resistant bacteria from cattle represent a substantially lower risk to human health than those from poultry and pork.

According to data compiled from the NORM-VET surveillance programme, this also seems to be the case for Norwegian production animals. Meat from animal species other than poultry has not been systematically monitored. There is some information on the occurrence of resistant indicator bacteria in pork and minced meat from cattle and mutton from the early years of the NORM-VET programme. These data demonstrated a low occurrence of resistant *E. coli* and enterococci. However, some multi-resistant isolates were detected (Sunde and Norström, 2006). These isolates usually exhibited resistance towards the "older" antimicrobial agents, such as streptomycin, sulphonamides, and ampicillin, and, to a certain extent, tetracycline (Sunde and Norstrom, 2006). Resistance to fluoroquinolones was very rare. In addition to poultry, investigation of ESBL/AmpC has been performed on faecal samples from pigs using a selective method, and only one ESBL/AmpC-positive finding from pigs has been recorded to date (NORM/NORM-VET, 2014).

Very few of the bacteria in fish and/or fish products are zoonotic agents, and all investigated cases of quinolone resistance have been associated with chromosomal mutations, i.e., not linked to mobile genetic elements. Thus, the risks from such resistance determinants to human health are considered negligible.

Our knowledge regarding transmission of resistant bacteria from fresh produce and dairy products to humans is limited. Exposure via imported products, in particular from fresh produce, is probably larger than the exposure via domestic products in Norway.

Overall, food processing probably contributes to a reduction in the exposure of consumers to resistant bacteria.

6.3.1 LA-MRSA

LA-MRSA may colonize humans that have direct contact with pigs, or the bacteria may enter the food chain during slaughter and become transferred to pork products (Normanno et al., 2015). Transmission of MRSA CC398 from livestock to humans occurs predominantly in people with occupational contact with animals. Spread of MRSA CC398 to household members of these persons has also been observed frequently, but dissemination in the general population is limited to date. However, in areas with intensive livestock husbandry, about 20-38 % of MRSA CC398 cases among humans cannot be epidemiologically linked to direct livestock contact, indicating other transmission pathways (Kock et al., 2014). The foodborne transfer of LA-MRSA currently seems to be of minor importance in Europe. In Denmark, the proportion of LA-MRSA isolated from humans with MRSA-infection has increased considerably during the last five to ten years. A similar tendency is also reported

from the Netherlands. This suggests that the prevalence of LA-MRSA in livestock has an impact on human MRSA epidemiology in countries with extensive pig production. In the period 2007-2013, Statens Serum Institut (SSI) in Denmark received a total of 8,375 MRSA isolates for characterisation, and 15 % of them belonged to CC398, which can be transmitted from pigs to humans and vice versa. Both the number and proportion of CC398 cases increased during the period. Thus, only 2 % of cases were CC398 in 2007, whereas this share had increased to 31 % in 2013. The number of persons positive for MRSA CC398 continued to increase in 2014 (NFI and SSI, 2014).

The *mecA* gene, located on the mobile SCCmec, may be disseminated between staphylococcal species, both coagulase-positive and coagulase-negative. So far, transfer of *mecA* to bacterial species other than staphylococci has not been reported.

The origin of LA-MRSA in the various cases among Norwegian pigs is believed to be introduction via foreign workers. The epidemiological shift involving transmission of MRSA from humans to animals, and back from animals to humans, is a matter of public health concern. However, transfer of MRSA (direct transfer) or *mecA* (indirect transfer) to humans via food consumption has never been identified, neither internationally nor in Norway. Although animals colonized with MRSA play a role in the transfer of MRSA to farmers and their household members, and the transmission of MRSA to humans due to consumption of food is not documented, the dynamic of that transmission may change due to evolutionary processes that allow the strain to adapt to a human lifestyle. The transfer of MRSA via raw food due to “poor” kitchen hygiene cannot be discounted. Special attention should also be paid to persons in close contact with animals colonized by LA-MRSA, in particular when they work as health workers or are admitted to hospitals as patients.

6.3.2 Quinolone-resistant bacteria

As campylobacteriosis is the most frequently reported foodborne zoonosis, both in Norway and in other developed countries, quinolone-resistant *Campylobacter* may represent a direct hazard to human health. A Norwegian study on the occurrence of AMR in *Campylobacter* spp. revealed a very low occurrence of AMR in *C. jejuni* from broilers and broiler house environments. Quinolone resistance was not observed, although quinolone-resistance rates of approximately 4 % are reported in *C. jejuni* from domestically acquired human cases (Norstrom et al., 2007).

The NORM-VET reports for 2013 and 2014 documented a high prevalence of quinolone-resistant *E. coli* (QREC) in poultry products (chicken and turkey). The Veterinary Institute reports (VI, 2015) that they have reason to believe that a similar prevalence has occurred in these products since 2000. The occurrence of QREC in poultry products may represent an indirect hazard to humans, as intestinal *E. coli* may become opportunistic pathogens and cause disease in other organs than the intestine. Most UTI are, for example, caused by *E.*

coli and, in Norway, fluoroquinolone-resistant *E. coli* as a cause of UTI is regarded as an emerging problem. However, it is unknown whether, or to which degree, QREC of poultry origin are able to colonize human intestines and become established as part of the resident intestinal microbiota. Furthermore, it is not known whether poultry QREC are able to cause UTI in humans. However, genetic similarities between avian pathogenic *E. coli* and human uropathogenic *E. coli* (UPEC) have been demonstrated (Mora et al., 2013). A study from Iceland demonstrated that genetically related resistant *E. coli* could be isolated from poultry meat produced in Iceland and from humans in Iceland, demonstrating that poultry can be a source of quinolone-resistant *E. coli* in humans (Thorsteinsdottir et al., 2010). Poultry products are heat-treated before consumption, either at food-processing plants or at home, and this treatment will kill the vast majority of the bacteria. Whether fragments of released bacterial DNA containing resistance genes on the surface of, for example, chicken fillets, can become transformed into other live intestinal bacteria and then expressed, is currently only a theoretical scenario.

6.3.3 ESBL / AmpC

Resistance genes mediating resistance to 3rd and 4th-generation cepahlosporins are usually located on mobile genetic elements like plasmids, and can easily be transferred and recombined among Gram-negative species like *E. coli*, *Citrobacter*, *Enterobacter*, *Klebsiella,* and other possibly pathogenic bacteria common to animals and humans *(Bfr-2015*). A study from the Netherlands demonstrated a high degree of similarity between resistance genes and MLST patterns of *Enterobacteriaceae* from meat and from human patients, suggesting a possible relationship between contamination of meat with drug-resistant bacteria and presence of specific genes in humans (Overdevest et al., 2011). It is not known whether the level of cephalosporin-resistant *E. coli* detected in poultry products produced in Norway represents a threat to public health. A recent report from Sweden concluded that the transmission of ESBL/cephalosporin-resistance from a food reservoir seems to be limited (Egervarn et al., 2014).

The origin of the ESBL/AmpC-producing bacteria in broiler production in Norway is believed to be via import of breeding material (Mo et al., 2014). Other possible explanations are transmission from humans, or introduction via water and/or feed. *bla*$_{CTX\text{-}M\text{-}14}$ and *bla*$_{CTX\text{-}M\text{-}15}$ are the predominant ESBL/AmpC-encoding genes in *Enterobacteriaceae* that have been isolated from human infections, while the pAmpC bla$_{CMY\text{-}2}$ is the predominant cepholosporin-resistance encoding gene from food-producing animals (poultry) (Mo et al., 2014). However, a recent study identified similar genotypes of *E. coli* producing beta-lactamase C (AmpC bla$_{CMY\text{-}2}$ variants) in humans and poultry (Berg et al., 2015). The probability of transmission from a poultry reservoir to humans is, nevertheless, rare, as the prevalence of human clinical *E. coli* isolates with characteristics resembling those from the poultry reservoir is very low.

Carbapenemase-producing Gram-negative bacteria: to the best of our knowledge, no acquired carbapenemase-producing (class B beta-lactamase) Gram-negative bacteria have been reported in food-producing animals or food in Norway.

6.3.4 VRE

E. faecium is highly clonal in its structure, and strains of vancomycin-resistant *E. faecium* are predominantly host-specific. Strains isolated from hospitalized patients are genetically different from strains prevailing in the faecal flora of non-hospitalized persons (Willems et al., 2000). A common Tn*1546* reservoir, which is a carrier of the *vanA* gene, is probably accessible to a variety of *E. faecium* recipients.

6.4 Negligible vs non-negligible probability

The probability for transfer of antimicrobial resistant bacteria or resistance genes associated with food in Norway has been summarised in Table 6-1. Based on available data on the occurrence of resistant bacteria or AMR genes in food-producing animals, foods of animal or plant origin, and water, the probability has been classified as negligible or non-negligible, according to the following definitions:

- Negligible – the probability of transfer of AMR is extremely low. A negligible probability should be considered insignificant.
- Non-negligible – the probability of transfer of AMR is greater than negligible. The non-negligible probability should be considered significant, but it is currently not possible to distinguish between different levels.

Information obtained from antimicrobial surveillance studies is important for establishing trends in pathogen AMR and for identifying emerging pathogens at the national and global levels. This information enables the development of targeted approaches to help control AMR (Masterton, 2008). AMR surveillance requires development of tools and standards for harmonized surveillance in humans, and for integrating that surveillance with surveillance of AMR in food-producing animals and food chains (WHO, 2014). Surveillance data are lacking regarding AMR for different food and food chains, in particular imported foods, in Norway. This lack of such data has made it difficult to reach any firm conclusions regarding the probability of AMR transmission from food to humans in Norway. It is also difficult to rank the probabilities with regard to relative importance.

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6.5 Summary of probability characterization

Table 6-1. The probability of AMR transmission from Norwegian food chains to humans. The classification is based on the VKM's expert opinion.

	Negligible		Not negligible		Comment (see Data gaps and Uncertainties)
	Documented	Insufficient documentation	Documented	Insufficient documentation	
Pigs					
Live animals			x		LA-MRSA: Low prevalence limits transmission of LA-MRSA between live animals.
Food processing		x			
Pork		x			
Poultry					
Live animals			x		Mostly ESBL/AmpC and QREC
Food processing				x	Data gaps
Meat			x		Mostly ESBL/AmpC and QREC
Eggs		x			
Cattle					
Live animals	x				Data for 2015 until August
Food processing		x			
Beef	x				Data for 2015 until August
Milk/dairy products	x				Pasteurised milk: no data for dairy products from unpasteurised milk
Fish					
Live animals	X				

	Negligible		Not negligible		Comment (see Data gaps and Uncertainties)
	Documented	Insufficient documentation	Documented	Insufficient documentation	
Food processing	x				
Fish products		x			
Seafood					
Live animals		x			
Food processing		x			
Seafood products		x			
Fresh produce		x			
Drinking water		x			

7 Risk-reduction measures

7.1 Recognised measures

Measures with the potential to reduce the risk arising from resistant microorganisms can be implemented at the primary production level, further down the line in food processing, and at the consumer level.

7.1.1 Primary production

Several international institutions have issued recommendations for measures to reduce the risk of development and/or transfer of AMR at primary production level (CAC, 2005; IFT, 2006; WHO, 2011).

Codex Alimentarius (2005) focused on antimicrobial use in food-producing animals, while recognizing that AMR is also an ecological problem and that management of AMR may require addressing the persistence of resistant microorganisms in the environment. Their recommendations include the reduced and responsible use of antimicrobial agents in food-producing animals through improving animal health, limiting and controlling the use of antimicrobials by the veterinary profession or other parties with the required expertise, and banning the use of antimicrobials as growth promoters.

WHO (2011) has developed policies for reduced need for, and prudent use of, antimicrobials in animal husbandry. The WHO Global Strategy for Containment of Antimicrobial Resistance includes recommendations for interventions to reduce the overuse and misuse of antimicrobials in food-producing animals for the protection of human health, based on the WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food.

IFT (2006) states that those who control or administer antimicrobial use in human medicine, veterinary medicine, and production agriculture can have the greatest impact in controlling resistance. In human medicine, appropriate therapy and use of improved patient diagnostics and treatments minimize resistance selection. In veterinary medicine and agricultural production, the implementation of various management strategies (such as responsible use guidelines, quality assurance programmes, and antimicrobial alternatives), coupled with government regulations, should decrease the opportunities for selection of antimicrobial resistant microorganisms.

SAFEFOOD (2010) and EFSA (2008) suggest solutions in the prudent use of antimicrobial agents, and they recommend surveillance and further research.

7.1.2 Food processing

To our knowledge, codes or policies such as those mentioned above have not been recommended for food processing. This is probably because resistance in the food chains mostly originates at primary production.

In addition to general hygiene measures that should be observed in food processing, the use of steam or hot water for decontamination at slaughter has been shown to reduce the overall load of bacteria on carcasses (VKM, 2010). As this measure does not involve use of chemicals it is unlikely to induce AMR, but has the potential to reduce the transfer of AMR. However, closer assessment on the extent of this potential requires further research.

Food processing generally is likely to reduce the level of resistant bacteria on products. Moreover, the impact of disinfection agents on reducing AMR in the food chain is most likely much larger than the potential for spread of resistant clones due to co-resistance or cross-resistance. Consequently, the introduction of further measures, in addition to those already in place, to reduce human exposure at the food-processing stage are unlikely to have a significant impact. Recontamination of products from food handlers may occur, but the frequency of such exposures is unknown. It is therefore difficult to assess the risk reduction potential of further measures during food processing.

7.1.3 Consumer level

Risk-reduction measures preventing transfer of microorganisms at the consumer level are important, regardless of whether the microorganisms are resistant. Thus, information on levels of risk associated with specific foods, e.g. raw meats, and recommendations for safe handling are important. However, it is difficult to assess the magnitude of risk reduction that is achieved by measures implemented at the consumer level.

7.2 Norwegian perspective

One of the most important measures for reducing antimicrobial use, as a major driver of AMR, is “disease prevention”, in particular in intensified animal production. For many years prudent use of antimicrobial agents has been an important measure that has contributed to the low prevalence of AMR in food-producing animals in Norway.

The use of antimicrobial agents in primary production in Norway has been reduced considerably over the last two decades, both in terrestrial animals and in aquaculture. Thus, risk-reduction measures of prudent and controlled use of antimicrobials, as recommended by international institutions, are already in place in Norway.

At the food-processing level, the implementation and monitoring of proper hygienic measures are imposed through the Food Law. If those measures are upheld, the level of resistant bacteria on products is likely to be reduced. Thus, introduction of additional measures is not likely to decrease contamination levels further.

Risk-reduction measures at the consumer level, including information on levels of risk associated with specific foods or food categories, and recommendations for safe handling (e.g. kitchen hygiene), are important. However, the magnitude of risk reduction achieved by such measures is difficult to assess.

Possible reductions of AMR in bacteria due to anticoccidial agents has been discussed in a separate risk assessment (VKM, 2015b).

Poultry production in Norway is dependent on import of grandparent and/or parent animals for breeding, as well as hatching eggs. Resistant bacteria have probably been introduced via such imports. It is possible to reduce the risk by introducing animals that are certified as being “free” of antimicrobial resistant bacteria.

8 Uncertainties

The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions identified in all the previous steps. Discrimination between uncertainty and variability is important in subsequent selection of risk management options. Biological variation includes the differences in virulence that exist in microbiological populations and variability in susceptibility within the human population and particular sub-populations. (http://www.fao.org/docrep/005/y1579e/y1579e05.htm). According to EFSA's guidance regarding uncertainties: assessments must state clearly and unambiguously the uncertainties that have been identified and their impact on the overall assessment outcome.

In this assessment, a number of uncertainties related to the probability of transmission of AMR from the food chains to humans have been identified. Many of these uncertainties may overlap with the data gaps (Section 10).

The uncertainties identified are as follows:

- Lack of knowledge on the extent to which resistance genes from dead bacteria in food, including from pasteurised milk and milk products, can be taken up and integrated into the genome of other resident gut bacteria (transformation).
- The proportions of ESBL/AmpC-*E. coli* and QREC are currently lower than those of non-ESBL/AmpC and quinolone-susceptible *E. coli* in poultry products. However, the colonization rates of resistant/susceptible isolates in humans are unknown. There are uncertainties regarding the ability of these bacterial strains to colonize humans and in the ability of their AMR genes to be transferred to resident bacterial species in humans.
- Animal-to-human transmission of AMR requires greater understanding of the genetic interactions and spread that occur in commensal and environmental bacteria.
- AMR is an evolving situation. Those factors that may promote/reduce the transmission of antimicrobial resistant bacteria and their corresponding gene determinants have not been identified.
- Transmission of LA-MRSA to humans due to consumption of food has not been reported to date. However, the presence and transmission of methicillin-resistant coagulase-negative staphylococci from pigs/pork to humans have not been studied and identified.
- A possible link between resistance against narasin and vancomycin has been identified in enterococci. The ability of these isolates to colonize humans and transfer their resistance genes to other intestinal bacteria in human is unknown.
- For uncertainties regarding laboratory methods, see Appendix 3.

9 Conclusions (with answers to the terms of reference)

1. **Which strains of bacteria/resistance genes are/will be of most importance concerning transfer from the food chain (directly from any part of the food chain and from the food itself) to humans in Norway?**

    a. Pig production and pork products

The most important resistant bacteria/AMR determinants from pig production and products are LA-MRSA.

Environmental contamination with LA-MRSA plays an important role in the acquisition of these bacteria in farmers, farm workers, and their household members. The probability of transfer to humans in direct contact with pigs is considered **non-negligible.** The other AMR forms assessed in this report are considered to be **negligible**.

The transmission of LA-MRSA to humans due to consumption of food has not been reported to date. Moreover, the low prevalence and the extensive measures that have been implemented make such transmission unlikely. Therefore, the probability of transfer to humans either in direct contact during food processing or during preparation and consumption of pork products is considered **negligible.**

    b. Poultry and egg production and products

The most important resistant bacteria/antimicrobial resistant determinants from poultry and poultry products are ESBL/AmpC-producing bacteria and the corresponding genes.

Environmental contamination with ESBL/AmpC-producing bacteria and their corresponding genes, as well as quinolone-resistant bacteria like QREC and corresponding genes, may play an important role in the acquisition of these bacteria in farmers, farm workers, and their household members. Data regarding such transmission is lacking. The probability of transfer of QREC and ESBL/AmpC-producing bacteria and corresponding genes to humans from live poultry via food processing and from preparation of poultry meat is considered **non-negligible**. Such transfer has been identified and reported internationally, although the incidence so far is not high. The other antimicrobial resistance forms assessed in this report are considered to be **negligible**.

The probability of transfer of AMR to humans through the consumption of eggs is considered **negligible**.

Cattle production and products

Based on available data, knowledge and occurrence of antimicrobial resistant bacteria in cattle in Norway, the probability of transfer of MRSA, VRE, and ESBL/AmpC-resistant bacteria/antimicrobial resistant determinants via live animals, food processing, beef, and milk is considered **negligible**.

Aquaculture and aquaculture products

Due to the limited use of antimicrobial agents and the low prevalence of antimicrobial resistant bacteria, the probability for transfer of antimicrobial resistant bacteria/AMR determinants to humans through aquaculture and the aquaculture chain is considered to be **negligible**. In particular it should be emphasised that bacteria from fish and bacteria adapted to humans have different optimal temperatures for growth, and psychrophilic marine bacteria in Norwegian aquaculture seldom colonize humans.

Our knowledge about AMR profiles in fish and fish products and other seafood imported to Norway is limited.

Fresh produce (fruit, berries, and vegetables)

Fresh produce is susceptible to contamination by antimicrobial resistant bacteria/AMR determinants in the form of commensals or foodborne pathogens, either from the production environment (i.e. soil, irrigation water, manure, wild animals) or as a result of human handling from farm-to-fork. The probability for transmitting antimicrobial resistant bacteria or AMR genes to humans via fresh produce produced in Norway is considered **negligible**. The prevalence of resistant bacteria is considered to be higher in imported products of plant origin than in domestic products. The probability for transmitting antimicrobial resistant bacteria or AMR genes to humans via imported fresh produce is considered **non-negligible**.

Systematic surveillance for bacterial contamination of fresh produce in Norway has not been established for either domestic or imported products.

Drinking water

The probability of transfer of antimicrobial resistant bacteria or AMR determinants through drinking water is difficult to assess. Data on the prevalence and enumeration of enteric pathogens in surface water, groundwater, or groundwater under the direct influence of surface water, are sparse. Furthermore, there are very little data on the concentrations of bacterial pathogens in groundwater under non-outbreak conditions. However, lack of data does not preclude that such transmission occurs. In this assessment, we have evaluated the probability of transfer of antimicrobial resistant bacteria or AMR determinants to human from drinking water in Norway as being **negligible**.

2. Where and how are resistant bacteria and/or antimicrobial resistance introduced to, or induced in, the food chain, and how are they transferred through the food chain and to humans?

Only AMR defined as non-negligible in the answer to question 1 are considered here.

a. Pig production and pork products

Occurrence of LA-MRSA in Norwegian pig production has been detected since 2011. It is believed that LA-MRSA was introduced to pig herds via foreign workers, and has spread to new herds through animal trade and people.

People with direct contact with animals, like farmers, farmworkers, and veterinarians, have been found to carry LA-MRSA of the same genotype as the animals to which they have been exposed.

A similar scenario to the situation regarding live animals may occur if workers from countries in which MRSA (LA-MRSA and/or CA-MRSA) have reached high endemic levels work in food processing plants. However, pig products are not consumed raw, and most food production measures will reduce the load of microbes in the product. Nevertheless, the risk for transfer of MRSA via raw pork due to poor kitchen hygiene cannot be discounted.

b. Poultry and egg production and products

Occurrence of ESBL/AmpC-producing *E. coli* in Norwegian poultry production has been detected since 2006. It has now been demonstrated that broiler production in Norway has a high prevalence of *E. coli* resistant to 3rd-generation cephalosporins. Imported breeding animals have been shown to be carriers of ESBL/AmpC-producing *E. coli*, and are regarded as the probable origin of the resistant bacteria. Furthermore, the prevalence of QREC is high in Norwegian poultry products. At present, it seems that quinolone-resistance in *E. coli* is mainly chromosomally mediated and not harboured on plasmids. However, chromosomal resistance genes can also be transferred to other bacterial species through transformation.

The high prevalence of antimicrobial resistant bacteria in live animals is reflected in a high prevalence in broiler meat also. Although broiler products are not consumed raw, and most food production measures will reduce the load of microbes in the product, a relatively high proportion of poultry products are sold as fresh meat, frequently in modified atmosphere. The risk of transfer of ESBL/AmpC-producing *E. coli* via these products due to poor kitchen hygiene cannot be dismissed.

c. Fresh produce

Fresh produce (vegetables, fruits, berries and other foods of plant origin) can be contaminated with AMR at any stage of the farm-to-table continuum, but, in particular, through fertilization with manure or sludge, through irrigation, and through handling. As emphasized in the answer to question 1, the prevalence of AMR is considered to be higher in imported products than in domestic products.

Many of these products are consumed without prior processing or only minimal processing, and consequently the microbial load on the products is not reduced before consumption, or only to a minor degree.

3. To what extent will exposure through the food chain contribute to the total load on human health of each of the most important resistance forms?

Due to insufficient of data, it is impossible to calculate the relative importance of antimicrobial resistant bacteria originating from the food chain as compared with the total load of bacterial resistance to which humans are exposed. Thus, the impact on human health cannot be estimated.

There are many barriers in the food chain, particularly during food processing and cooking, and these will make a substantial contribution towards preventing, or at least reducing, the transmission of antimicrobial resistant bacteria from food to humans.

10 Data gaps

10.1 Data gaps identified for all food chains include:

- Data regarding the vast reservoir of AMR in the environmental, animal, and food reservoirs are lacking.
- Data regarding the routes and frequencies of transmission of AMR from live food-producing animals and foodstuffs of different origins to humans and vice versa are lacking.
- Data on susceptibility-testing of bacteria isolated from the domestic food chains are limited.
- Data enabling comparisons of genetic determinants of AMR of bacteria such as *Enterococcus, Salmonella, Campylobacter, and E. coli*, isolated from human patients and those recovered from foods and animals are limited.
- Data regarding transformation of AMR genes via food to bacteria in humans are lacking.
- Data regarding the relative importance of antimicrobial resistant bacteria transmitted through food, as compared with anaerobic and non-culturable bacteria in animals and humans, are lacking.
- The impact on human health of antimicrobial resistant bacteria transferred through food has not been evaluated.
- The prevalence of LA-MRSA in humans in Norway is not known.
- Information regarding transformation of AMR genes from food to human microbiota is lacking.
- Data on AMR source attribution are lacking.
- Although, there are some studies regarding biocide resistance in bacteria originating from food, there is a lack of monitoring information and knowledge about the clinical impact in humans is lacking.

10.2 Data gaps identified in food chains

10.2.1 Pig production and pork products

- Antimicrobial susceptibility data, in particular against methicillin in CoNS originating from pigs, are lacking.

10.2.2 Poultry and egg production and products

- The effects of narasin used as anticoccidal agents in poultry on intestinal bacteria other than enterococci are not known.

- Although data regarding possible cross-resistance between narasin and bacitracin and between narasin and vancomycin are available, data regarding colonization of such bacteria in human microbiota are lacking.
- Data regarding transient or permanent colonization of QREC, VRE, narasin-resistant enterococci, and ESBL/AmpC-producing bacteria in humans are lacking.

10.2.3 Bovine production and products

- Resistance testing of bacteria causing mastitis in Norway, in particular staphylococci, is performed only for susceptibility towards penicillin.
- Data regarding antimicrobial resistant bacteria isolated from milk and milk products should be updated.

10.2.4 Aquaculture and aquaculture products

- Knowledge regarding antimicrobial resistant bacteria among imported aquaculture products is limited.

10.2.5 Fresh produce (fruit, berries, and vegetables)

- Little is known about non-animal sources of AMR (e. g. fresh produce, wastewater).

10.2.6 Water

- Antimicrobial susceptibility data from bacteria isolated from drinking water in Norway are lacking.
- Data are sparse on the concentrations of microbial pathogens in groundwater in Norway under non-outbreak conditions.

10.2.7 Imported food

- Susceptibility data regarding bacteria isolated from imported food are lacking.
- Information regarding antimicrobial resistance in pathogenic and non-pathogenic bacteria from imported food is lacking.
- There are no data available on the occurrence of resistant bacteria in Norwegian or imported milk products.

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12 Appendix I – tables

Table 12-1. WHO listing (3rd revision, 2012) of antimicrobials for human medicine (Doyle et al. 2014)

Critically important	Highly important	Important	Unclassified
Aminoglycosides	Amdinopenicillins	Aminocyclitols	Ionophores
Carbapenems and other penems	Amphenicols	Cyclic polypeptides	Bambermycins
Cephalosporins (3rd and 4th generation)*	Cephalosporins (1st and 2nd generation)	Nitrofurantoins	Carbadox
Cyclic esters	Licomsamides	Nitroimidazoles	
Fluoro and other quinolones*	Penicillins (anti-staphylococcal)		
Glycopeptides*	Pleuromutilins		
Glycylcyclines	Riminofenazines		
Lipopeptides	Steroid antibacterials		
Macrolides and ketolides*	Streptogramins		
Monobactams	Sulfonamides		
Oxazolidinones	Sulfones		
Penicillins (natural aminopenicillins and antipseudomonal)	Tetracyclines		
Polymyxins			
Rifamycins			
Tuberculosis and other mycobacterial drugs			

*The top 4 critically important antimicrobials are prioritized based on: (1) high absolute number of people affected by diseases for which the antimicrobial is the sole or one of few alternatives to treat serious human disease, and (2) high frequency of use of the antimicrobial for any indication in human medicine, since usage may favor selection of resistance. In addition, a focusing criterion for the above classifications is that there is a greater degree of confidence that there are nonhuman sources that result in transmission of bacteria or their resistance genes to humans (WHO 2012).

Table 12-2 Antibacterial agents, marketed (Y) or sporadically approved on special licence (N) in Norway, for therapeutic and prophylactic use in livestock including fish.

Antimicrobial class	Antibacterial agent	Marketed	Animals use[a]	Human use	Antimicrobial agents used in human medicine - WHO definition[d, e, f]	Comment
β-lactams	Amoxicillin	Y	C, S, P	Y	d	
	Benzylpenicillinprocaine	Y	C, S, Sh, G		d	
	Phenoxymethylpenicillin	N	P	Y	d	
β-lactams + clavulanate	Amoxicillin + clavulanate	Y	C, S	Y	d	
β-lactams, combinations	Benzylpenicillinprocaine + Dihydrostreptomycin	Y	C, S, Sh, G	Y	d+e	
Aminoglycosides	Dihydrostreptomycin	Y	C, S, Sh	Y	e	
	Gentamicin	N	C	Y	d	Limited use in animals
	Neomycin	Y	C, S	Y	e	Only young animals
Tetracyclines	Doxycycline	Y	P, S	Y	e	
	Oxytetracycline	Y	C, S, Sh, P, F	Y	e	
Fluoroquinolones	Enrofloxacin	Y	C, S	N	-	b
	Flumequine	Y	F	N	-	b
	Oxolinic acid	Y	F	N	-	b
Phenicol	Florfenicol	Y	C, F	N	-	b
Macrolides	Gamitromycin	Y	C	N	-	b
	Tylosin	N	S	N	-	b, Limited use in animals
Sulphonamides + trimethoprim	Trimethoprim/sulphadoxine	Y	C, S, Sh, G	Y Other combination used[b]	d	Trimethoprime+ sulphomethoxazole

Antimicrobial class	Antibacterial agent	Marketed	Animals use[a]	Human use	Antimicrobial agents used in human medicine - WHO definition[d, e, f]	Comment
	Trimethoprim/sulphadiazine	Y	C, S, Sh, G, F	See above	d	b, Sulphadiazine i used against colitis ulcerosa and sometimes against toxoplasmosis (in combination)
Ionophore	Narasin	Y	P	N	-	c
Pleuromutilins	Tiamulin	Y	S	N	-	g
Combinations	Benzylpenicillinprocaine + sulphadimidine + dihydrostreptomycin	Y	C, S	N	-	b, Intrauterine in animals
	Spectinomycin[h] + lincomycin 2+1	Y	F	Y[h]	e	b

a- C cattle; P poultry; S swine; Sh sheep; G goat, F fish

b- Closely related analogues are used in, and are of importance to, human medicine.

c- Narasin is patented as an antibiotic, marketed as feed additive and not as a therapeutic agent in Norway.

d- Critically important antimicrobials

e- Highly important antimicrobials

f- Important antimicrobials

g- Not used in human medicine and no closely relate analogue used in human medicine.

h- Spectinomycin is used for treatment of gonorrhoea.

Veterinarians may also use other antimicrobial agents with no marketing authorization listed above (off-label use).

Table 12-3. Total sales in kg active substance of antimicrobial VMPs for therapeutic use in farmed fish in Norway in the period 2003-2013.

Group of substances	Active substance	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Tetracyclines	Oxytetracycline	45	9	8	0	19	23	40	10	1	1	0
Amphenicols	Florfenicol	154	111	202	302	139	166	303	275	336	191	300
Quinolones	Flumequine	60	4	28	7	18	1	1	0	0	0	0
	Oxolinic acid	546	1035	977	1119	406	681	926	308	212	1399	672
Combinations	Spectinomycin + Lincomycin (2+1)	0	0	0	50	66	70	43	57	0	0	0

Table 12-4 Percentage (%) of resistance in indicator bacterial isolates (*E. coli)* isolated from pigs and pork in Norway (2001-2011). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available

Substance	**Faeces**					**Meat**		
	2001 (n=93)	2004 (n=125)	2007 (n=198)	2008 (n=258)	2011 (n=192)	2000 (n=158)	2002 (n=137)	2004 (n=97)
Tetracycline*	6.5	9.6	9.1	4.3	9.4	10.1	4	8.3
Chloramphenicol	2.2	0.8	0	0	0.5	3.8	0	1.0
Florfenicol	0	0	0	0	0	ND	0	0
Ampicillin	4.3	8.0	10.1	4.3	7.8	9.5	0.7	9.3
Cefotaxime	ND	ND	0.5	0	0.5	ND	ND	ND
Ceftazidime	ND	ND	ND	ND	0.5	ND	ND	ND
Sulphamethoxazole*	10.8	12.0	12.6	6.6	10.4	21.5	11.0	11.3
Trimethoprim	7.5	4.0	7.1	3.5	7.3	9.5	1.5	7.2
Gentamycin	0	0	0	0	0	0.6		1.0
Streptomycin	23.7	27.2	24.2	19.0	17.2	19.0	20.7	16.5
Kanamycin	ND	ND	1.0	0.8	1.6	3.2	ND	ND
Ciprofloxacin*4	0	0	0.5	0	0	0	0	0
Nalidixic acid	0	0	0.5	0	0	0	0	0
Colistin	ND	ND	ND	ND	ND	ND	ND	ND
ESBL blaTEM 52	ND	ND	ND	ND	0.5	ND	ND	ND

*sulphonamides before 2001, oxytetracycline before 2005, and enrofloxacin before 2006.

Table 12-5 Percentage (%) of resistance in indicator bacterial isolates (*E. coli*) isolated from broilers (breeders in 2012) and broiler meat in Norway (1999-2012). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available

Substance			**Faecal samples**				**Meat samples**				
	2002 (n=141)	2004 (n=86)	2006 (n=190)	2009 (n=162)	2011 (n=208)	2012# (n=113)	2000 (n=204)	2002 (n=155)	2004 (n=87)	2006 (n=119)	2012 (n=196)
Tetracycline*	12.0	7.0	3.7	8.0	4.3	5.3	11.8	5.0	6.9	5.0	2.0
Chloramphenicol	0	0.8	0	0	0.5	0	0	0	1.0	0	0
Florfenicol	0	0	0	0	0	0	ND	0	0	0	0
Ampicillin	13.5	17.4	13.2	11.7	15.4	15.0	10.8	7.1	23.3	7.6	5.6
Cefotaxime	ND	ND	1.1	0	1.0	0.9	ND	ND	ND	0	0.5
Ceftazidime	ND	ND	ND	0	1.0	2.7	ND	ND	ND	ND	1.0
Sulphamethoxazole*	17.0	14.0	8.9	7.4	9.6	8.8	32.4	15.5	12.6	6.7	7.7
Trimethoprim	0.7	2.4	3.1	2.5	5.8	4.4	7.8	1.9	2.3	0.8	2.6
Gentamycin	0.7	0	1.1	0	0	0	0.5	0	1.1	1.7	0
Streptomycin	5.7	2.3	2.1	3.1	4.8	1.8	7.4	3.2	5.7	7.6	3.1
Kanamycin	ND	ND	0	0	1.9	0.9	0	ND	ND	0	0
Ciprofloxacin*	0	0	1.1	8.0	1.9	0.9	0	1.2	0	0	2.6
Nalidixic acid	1.4	0	1.1	8.0	1.9	0.9	4.9	1.2	3.4	0	2.6
Colistin	ND	ND	ND	ND	0	0.9	ND	ND	ND	ND	0
ESBL_BLACMY2[#]	ND	ND	ND	ND	43.0	7.3	ND	ND	ND	ND	32.2

* oxytetracycline instead of tetracycline before 2005, sulphonamides instead of sulphamethoxazole before 2001, and enrofloxacin instead of ciprofloxacin before 2006.

#breeders

Table 12-6 Percentage (%) of resistance in indicator bacterial isolates (*E. coli*) isolated from turkeys and turkey meat in Norway (1999-2013). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available

Substance	Faecal samples		Meat samples	
	2007 (n=53)	2013 (n=109)	2007 (n=97)	2013 (n=154)
Tetracycline*	13.2	12.8	10.3	17.5
Chloramphenicol	1.9	0.9	1.0	2.6
Florfenicol	0	0	0	0
Ampicillin	15.1	12.8	13.4	23.4
Cefotaxime	0	0	0	0
Ceftazidime	ND	0	ND	0
Sulphamethoxazole	5.7	9.2	3.1	5.2
Trimethoprim	0	3.7	0	3.2
Gentamycin	0	2.7	1.0	0.6
Streptomycin	9.4	4.6	6.2	5.2
Kanamycin	3.8	0.9	0	1.9
Ciprofloxacin*	1.9	0.9	0	1.2
Nalidixic acid	1.9	0.9	0	1.2
Colistin	ND	0.9	ND	0
ESBL_BLACMY2[#]	ND	1.5	ND	2.6

* Oxytetracycline instead of tetracycline before 2005, and enrofloxacin instead of ciprofloxacin before 2006.

Table 12-7 Percentage (%) of resistance in indicator bacterial isolates (*E. faecium* and *E. faecalis*) isolated from broilers or layers (2013); (faecal samples or boot swabs) in Norway (2004-2013). Epidemiological cut-off values (ECOFFs) as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND= No data available, NR= Not relevant to test because of intrinsic resistance.

Substance	***E. faecium* / faeces**			***E. faecalis* / faeces**		
	2004 (n=62)	2006 (n=200)	2011 (n=176)	2004 (n=22)	2006 (n=5)	2011 (n=62)
Ampicillin	0	1.5	0.6	0	0	0
Bacitracin	18.0	41.0	38.1	9.1	20.0	19.4
Chloramphenicol	0	2.0	0	0	0	11.3
Erythromycin	6.4	18.5	5.7	9.1	20.0	25.8
Gentamycin	0	0	0	0	0	0
Kanamycin	ND	2.0	0.6	ND	0	0
Linezolid	ND	0	0	ND	0	0
Narasin**	79.0	72.5	68.8	22.7	20.0	4.8
Streptomycin	0	0.5	0.6	0	20.0	16.1
Tetracycline*	18.0	8.5	11.4	22.7	0	45.2
Vancomycin	4.8	0	0	0	0	0
Virginiamycin	1.6	3.5	0	1.6	NR	NR

*Oxytetracycline tested instead of tetracycline before 2005

**Cut-off values as defined in NORM/NORM-VET report 2011

Table 12-8 Percentage (%) of resistance in indicator bacterial isolates (*E. faecium* and *E. faecalis*) isolated from layers in 2013; (faecal samples or boot swabs) in Norway (2013). Epidemiological cut-off values (ECOFFs) as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND= No data available, NR= Not relevant to test because of intrinsic resistance.

Substance	***E. faecium* / faeces**	***E. faecalis* / faeces**
	2013(n=103)	2013 (n=89)
Ampicillin	1.0	1.1
Bacitracin	2.9	3.4
Chloramphenicol	0	0
Erythromycin	29.1	10.1
Gentamycin	0	0
Kanamycin	0	0
Linezolid	0	0
Narasin**	1.9	1.1
Streptomycin	0	3.0
Tetracycline*	7.8	31.4
Vancomycin	0	0
Virginiamycin	5.8	NR

*Oxytetracycline tested instead of tetracycline before 2005

**Cut-off values as defined in NORM/NORM-VET report 2011. Layers are not provided with narasin as a feed additive.

Table 12-9 Percentage (%) of resistance in indicator bacterial isolates (*E. faecium* and *E. faecalis*) isolated from broiler meat in Norway (1999-2012). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available; NR= Not relevant

Substance	***E. faecium***		***E. faecalis***	
	2004 (n=50)	2006 (n=89)	2004 (n=29)	2006 (n=14)
Ampicillin	0	0	0	0
Bacitracin	54	37.1	31.0	0
Chloramphenicol	0	0	0	7.1
Erythromycin	24	21.3	17.2	14.3
Gentamycin	0	0	0	0
Kanamycin	ND	1.1	ND	0
Linezolid	ND	0	ND	0
Narasin*	84.0	68.5	6.9	7.1
Streptomycin	2.0	0	3.4	7.1
Tetracycline	6.0	11.2	35.0	14.3
Vancomycin	0	1.1	0	0
Virginiamycin	14.0	3.4	NR	NR

Table 12-10 Percentage (%) of resistance in indicator bacterial isolates (*E. faecium* and *E. faecalis*) isolated from turkeys and turkey meat (1999-2013). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available; NR= Not relevant

Substance	***E. faecium***			***E. faecalis***	
	faeces		meat	faeces	Meat
	2007(n=55)	2013(n=95)	2007 (n=47)	2013 (n=33)	2007 (n=25)
Ampicillin	10.9	4.2	4.3	0	0
Bacitracin	23.6	14.7	27.7	18.2	16.0
Chloramphenicol	0	0	0	3.0	0
Erythromycin	20.0	12.6	38.3	18.2	24.0
Gentamycin	0	0	0	0	0
Kanamycin	0	0	0	0	0
Linezolid	0	0	0	0	0
Narasin*	63.6	81.1	51.1	12.1	4.0
Streptomycin	1.8	1.1	0	3.0	4.0
Tetracycline	23.6	18.9	17.0	45.5	52.0
Vancomycin	0	0	0	0	0
Virginiamycin	3.6	1.1	2.1	NR	NR

*Cut-off values as defined in NORM/NORM-VET report 2011

Table 12-11 Percentage (%) of resistance in indicator bacterial isolates (*E. coli*) isolated from cattle (faecal samples and meat) in Norway (1999-2013). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available

Year	Faecal samples 2003 (n=120)	Faecal samples 2005 (n=98)	Faecal samples 2010 (n=209)	Meat 2005 (n=90)
Tetracycline*	5.0	1.0	1.9	2.2
Chloramphenicol	0.8	0	0	0
Florfenicol	0	0	0	0
Ampicillin	2.5	2.0	1.9	3.3
Cefotaxime	ND	ND	0.5	ND
Ceftazidime	ND	ND	ND	ND
Sulphamethoxazole	10.0	1.0	3.3	3.3
Trimethoprim	0	0	0	3.3
Gentamycin	1.7	0	0	
Streptomycin	12.5	7.1	9.1	7.8
Kanamycin	ND	ND	0	ND
Ciprofloxacin*	0	0	0	0
Nalidixic acid	0	0	0	0
Colistin	ND	ND	0	ND

*Oxytetracycline tested instead of tetracycline before 2005 and enrofloxacin instead of ciprofloxacin before 2006

Table 12-12 Percentage (%) of resistance in indicator bacterial isolates (*E. faecium* and *E. faecalis*) isolated from cattle (faceal samples) in Norway (1999-2013). Epidemiological cut-off values as defined in the NORM/NORM-VET report of 2013 were used to classify the resistance. ND=No data available; NR= Not relevant.

Substance	**Faeces**		**Meat**			
	E. faecium	*E. faecalis*	*E. faecium*		*E. faecalis*	
	2003 (n=5)	2003 (n=6)	2003 (n=18)	2005 (n=26)	2003 (n=90)	2005 (n=84)
Ampicillin	0	0	0	0	0	0
Bacitracin	0	0	5.6	19.2	2.2	6.0
Chloramphenicol	0	0	0	0	0	0
Erythromycin	20	16.7	5.6	0	1.1	4.8
Gentamycin	0	0	0	0	0	0
Kanamycin	ND	ND	ND	ND	ND	ND
Linezolid	ND	ND	ND	ND	ND	ND
Narasin**	0	0	0	0	0	0
Streptomycin	0	16.7	0	7.7	5.6	15.5
Tetracycline*	0	0	5.6	3.8	7.8	22.6
Vancomycin	0	0	0	0	0	0
Virginiamycin	0	NR	11.1	0	NR	NR

*Oxytetracycline instead of tetracycline in 2003.

**Cut-off values as defined in NORM/NORM-VET report 2011

13 Appendix II - Figures

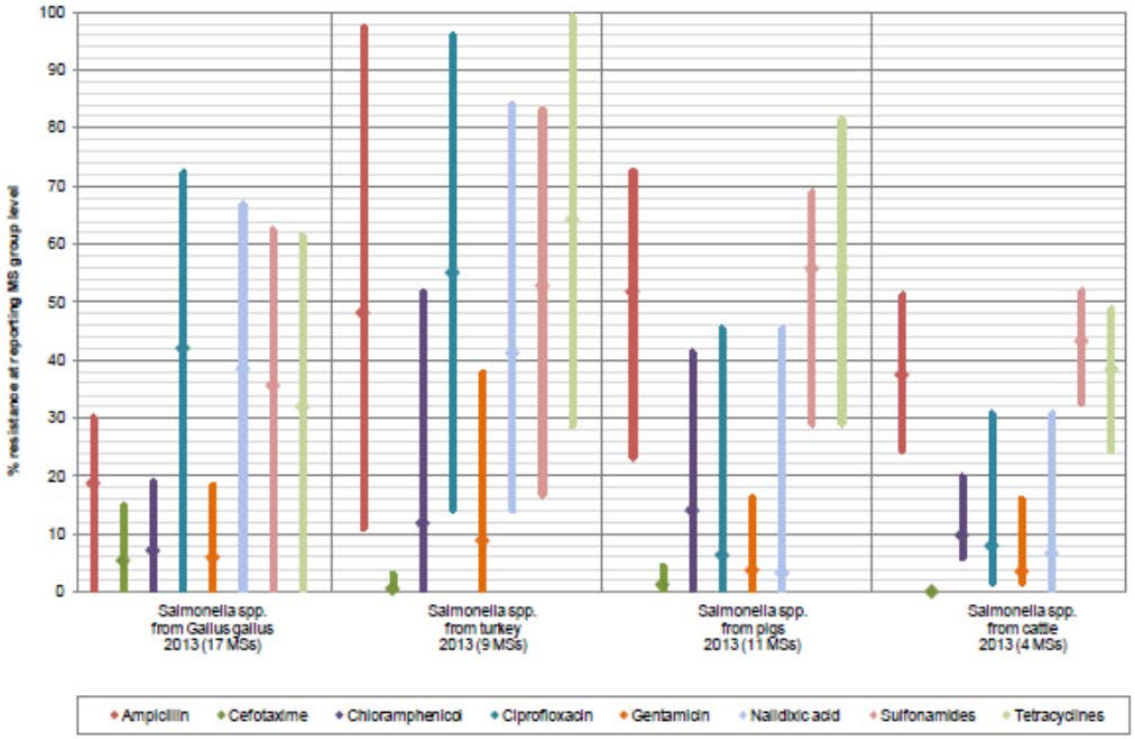

Figure 13-1. Occurrence of resistance to selected antimicrobials in *Salmonella* spp. from chicken, turkeys, pigs and cattle at reporting Member states group level in 2013 (EFSA/ECDC, 2015)

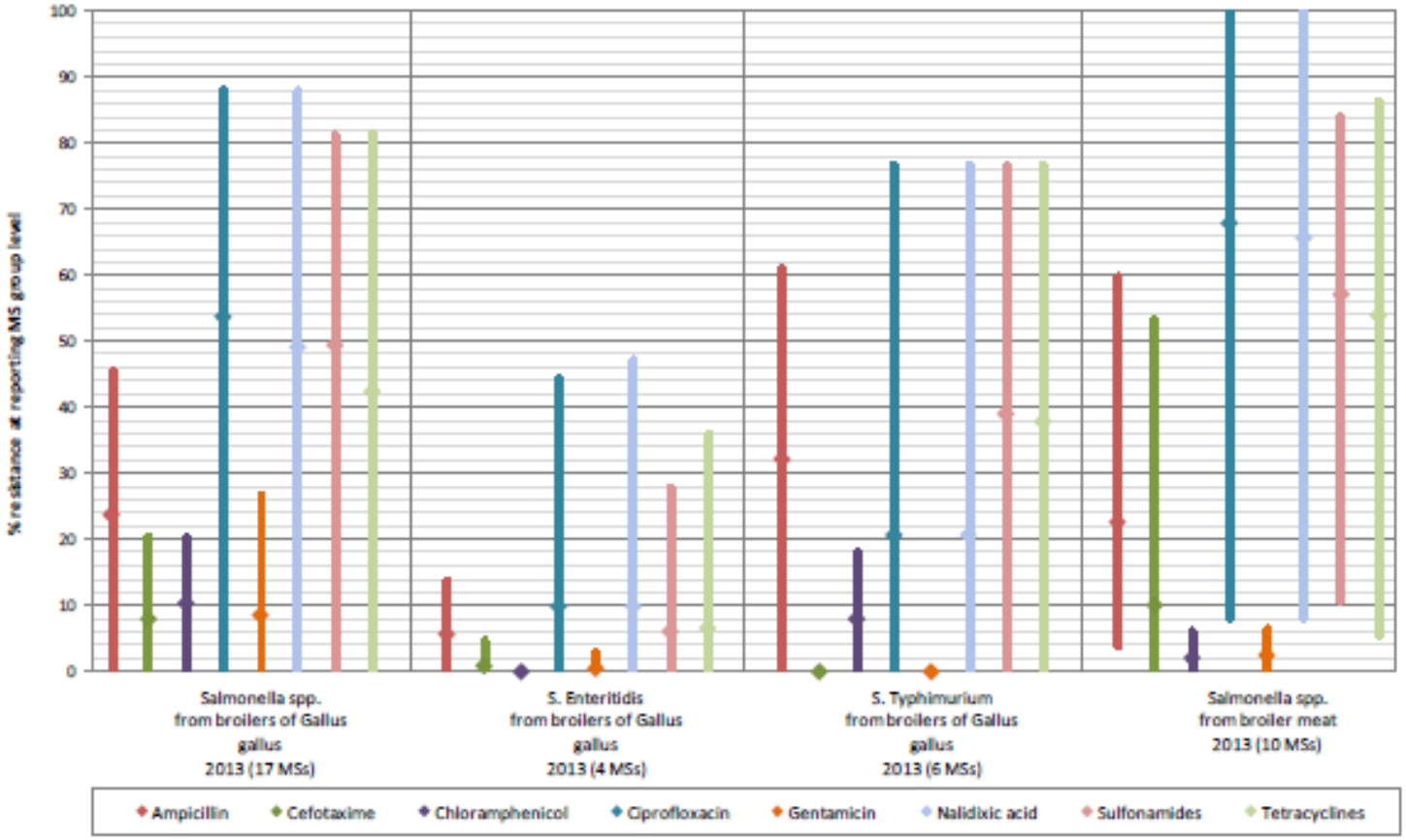

Figure 13-2. Occurrence of resistance to selected antimicrobials in *Salmonella* spp. from broilers of *Gallus gallus* and broiler meat, *Salmonella* Enteritidis and *Salmonella* Typhimurium from broilers at reporting Member states group level in 2013 (EFSA/ECDC, 2015)

14 Appendix III - Uncertainties - methodological aspects

Surveillance of AMR could either be passive or active, and the approach used will have a substantial influence on the prevalence of AMR measured. In passive surveillance the number of isolates depends on the samples submitted to the laboratories involved and it is possible that only samples from recurrent infections (already treated cases) or samples from specific regions of the country might be submitted. Active surveillance tries to collect samples representative for the whole country and with a sufficient sample size to enable comparisons over time. Monitoring the antimicrobial situation over time within the veterinary sector requires sampling from many different animal species and different kinds of food. The sample size needs to be high enough to enable comparison between the occurrence of resistance from year to year and also depends on the target size of the differences that should be detected. The occurrence of resistance might be clustered within farms or flocks, and to avoid this effect, only one sample per herd or flock should be included in the surveillance. One isolate of the monitored species is often picked at random from the agar and tested for susceptibility. The isolated bacterial species are normally tested for antimicrobial agents to which the “wild type” population are totally susceptible. The substances included in the test panels might not always be those used in veterinary medicine, but are included because of their importance for human health and as indicators for special resistance forms.

Different tests can be used for susceptibility testing: Minimum inhibitory concentration / disc diffusion tests / gradient tests. The results from the different approaches are not fully comparable. The platforms used should be standardised between laboratories in order to achieve the same results. Also the definition of resistance can differ according to the purpose of the susceptibility testing. Clinical breakpoints are used to define resistance in order to make the right decision regarding which specific antimicrobial agent should be used for therapy, whereas the epidemiological cut-off values could indicate emerging resistance in the bacterial populations. This is more thoroughly described in the NORM/NORM VET report 2013 and on the EUCAST homepage (www.eucast.org).

This results in an average prevalence of resistance for each bacterial species tested for each of the substances monitored in the country as a whole. However, as these isolates are chosen at random, resistant isolates might not be selected despite being present. Emerging resistances presenting at very low levels in the total bacterial flora might thereby not be detected.

In order to identify emerging resistances at an early stage it is possible to screen selectively for such resistances by using selective media. Only the isolates that are resistant will then grow on these media as the others will be suppressed. This methodology is much more sensitive for detecting low level, but emerging, resistances that are important to detect. However, the occurrence of such must be quantified in order to be able to reach conclusions on the burden of such emerging resistances.