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Risk profile on antimicrobial resistance

Risk profile on antimicrobial resistance transmissible from food animals to humans

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Colophon

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Photograph front page:
ESBL phenotypic screening test – A.H.A.M van Hoek

Summary

Since their introduction in the 1940s, antimicrobials have substantially reduced the human disease burden. As a side effect, their extensive use has resulted in selection and dissemination of antimicrobial resistant bacteria, which reduces the efficacy of initial treatment of infections and limits treatment options after diagnosis. In food animal production, antimicrobial drugs are widely used and antimicrobial resistance is increasing in both zoonotic and commensal bacteria. This has raised concerns about the risks of transmission of resistant zoonotic bacteria (direct hazards) and resistance genes (indirect hazards) from food animals to humans and the consequences for health care and public health. This report presents a risk profile to inform risk managers on the current knowledge on these potential health hazards as a first step in the risk management process. Two direct and one indirect hazard were selected to serve as examples in this risk profile, these are:

- quinolone-resistant *Campylobacter jejuni* (direct hazard);
- livestock-associated (LA-)MRSA (direct hazard);
- extended-spectrum beta-lactamase (ESBL-) producing bacteria (indirect hazards).

A summary of relevant information with respect to their risk for human health is given below.

Quinolone-resistant *Campylobacter*

In the last decades, quinolone resistance in *Campylobacter* isolates from poultry and human cases has increased strongly and was found to be directly related to the use of quinolones in poultry production. There is sufficient evidence of a causal relationship based on temporal, geographic, and epidemiological associations. Similar to sensitive bacteria, quinolone-resistant *Campylobacter* is mainly transmitted through consumption and preparation of broiler meat, but the role of other pathways is increasingly recognised. There are no indications that direct contact with food animals or handling meat poses a significant risk.

Campylobacter primarily causes acute gastroenteritis that usually is self-limiting; there are no indications that illness differs between cases with quinolone-resistant and susceptible strains. Antimicrobial resistance is not expected to increase the risk of chronic sequelae such as Guillain-Barré syndrome, reactive arthritis, irritable bowel syndrome and inflammatory bowel disease. Antimicrobial treatment is only indicated for severely diseased or immunocompromised patients. As a result of the high level of resistance in *Campylobacter*; quinolones are excluded for empiric treatment of gastroenteritis in primary health care as well as in hospitals. It is estimated that approximately 79,000 symptomatic *Campylobacter* infections occur annually and approximately 50% of these infections, i.e. 40,000 cases, are caused by quinolone-resistant strains. There are no indications that the disease burden has increased as a consequence of quinolone resistance and the healthcare costs are similar to those for susceptible *Campylobacter* infections. Social consequences and risk perception have not been studied.

Control of quinolone-resistant *Campylobacter* is similar to control of susceptible *Campylobacter* and may include control of infection in primary production combined with post-harvest measures, such as improved slaughter hygiene as well as scheduled processing combined with decontamination measures. Banning the use of fluoroquinolones in poultry may not solve the problem; in the USA such a ban has not resulted in reduced fluoroquinolone resistance in human clinical isolates in the first years after the ban. Several AMR-RAs are available for quinolone-resistant *Campylobacter* spp. They all show that (fluoro)quinolone use in the food animal reservoir contributes to resistance in humans, but the human health impact seems to be relatively small.

Livestock-associated MRSA (LA-MRSA)

LA-MRSA was first discovered in 2005 and is now spreading throughout the Dutch intensive livestock farming sectors. The majority of strains derived from food animals were of sequence type 398.There is sufficient evidence of a causal relationship between human clinical isolates and isolates from food animals based on temporal, geographic, epidemiological, and genetic associations. Direct contact with live food animals is the main transmission route from food animals to humans. Identified risk groups include pig and veal farmers and their families, as well as veterinarians and slaughterhouse workers. The carriage rate among pig and veal farmers in the Netherlands is approximately 30%; the risk of carriage increases with intensity and duration of animal contact. Person-to-person transmission is limited, which reduces the risk of secondary spread and limits hospital outbreaks. There are no indications that the presence of LA-MRSA on meat is a risk for public health or for food handlers.

LA-MRSA mainly causes skin or soft tissue infections, but is also capable of causing invasive infections. In recent years, approximately 100 LA-MRSA infections were reported annually, which constitutes 10-15% of all MRSA infections. Reported clinical cases of LA-MRSA concerned occupational groups in direct contact with livestock, patients with other underlying diseases, elderly patients, and patients that underwent surgery. LA-MRSA is multidrug resistant, which reduces treatment options. The disease burden and cost of illness (COI) of MRSA have not been quantified, but are likely to have increased with the rise of LA-MRSA infections. There are no indications that the individual disease burden of LA-MRSA differs from

HA-MRSA. MRSA patients are found to experience psychological problems due to stigmatization and isolation. Risk perception has not been studied.
Currently, there are no specific LA-MRSA control options outside healthcare, except for general initiatives in husbandry to reduce the use of antimicrobials. Potential control measures in primary production include purchase of MRSA-negative animals, restrictive use of antibiotics and thorough cleaning and disinfection; the effect of these measures is yet unquantified. Control options to prevent transmission to professionals include adaptations in animal production systems and the use of personal protection measures to reduce exposure to live farm animals and their environment. Personal protection measures are theoretically effective, but if not properly applied may increase the risk of colonization. There are no antimicrobial resistance risk assessments (AMR-RA) available for LA-MRSA; for most risk questions analytical epidemiological studies combined with microbial subtyping may suffice.

ESBL-producing bacteria

Extended-spectrum beta-lactamases (ESBLs) are enzymes that render Gram-negative bacteria resistant to beta-lactam antimicrobials and are inhibited by beta-lactamase inhibitors. They are an important reason for failure of initial cephalosporin treatment of infections caused by ESBL-producing bacteria. Since the beginning of this century, the number of ESBL-producing bacteria is increasing in human as well as veterinary isolates, in particular from poultry. The CTX-M type ESBL is currently most widespread among humans. ESBL-genes are usually located on plasmids that can be transferred between bacterial species. These plasmids often carry other antimicrobial resistance genes as well, rendering the bacteria multidrug-resistant.
There is sufficient evidence of an association between plasmids and the ESBL resistance genes they carry in human clinical isolates and in poultry isolates based on temporal, genetic, and epidemiological associations but the evidence is currently too limited to conclude about causal relationships. Recent data suggest that both foodborne transmission and direct contact play a role in the transmission from food animals to humans. In a pilot study, faecal carriage among Dutch broiler farmers was found to occur frequently (6 positive out of 18). The risk of the presence of ESBL-producing bacteria on meat for public health or professional food handlers is unclear. Extensive, laborious infection control measures are taken in the hospital setting to prevent dissemination of ESBL-producing bacteria. Alternative treatment options are limited, more expensive and may require hospitalization. It is estimated that in 2009, approximately 5400 urinary tract infections (UTI) cases in general practice patients were caused by ESBL-producing *E. coli*. In addition, there are an estimated 500 invasive infections by ESBL-producing bacteria reported in 2009. Knowledge on the risk factors for carriage and infection is limited. Identified risk factors are previous admission to health-care facilities, antimicrobial drugs usage, travelling to high-endemic countries and the presence of ESBL-positive family members. The disease burden and cost of illness have not yet been quantified, but are likely to be substantial and increasing. The social consequences and risk perception have not been investigated.
The voluntarily decision of Dutch veterinarians to stop using cephalosporins in poultry is the single current control option in place. Potential options in primary production are restrictive use of antibiotics and thorough cleaning and disinfection; the effect of these measures is yet unclear. In Canada, a temporary stop on using cephalosporins in poultry was effective in reducing the prevalence of ESBL-producing bacteria on poultry meat. Conductance of an AMR-RA would be helpful in addressing the problem of ESBL-producing bacteria, but has not been performed yet.

Recommendations

Based on the information collected, the following recommendations are made:

- to refine or make better use of the current human surveillance systems to enable monitoring of microbial resistance hazards attributed to the food animal reservoir;
- to initiate research to fill in the identified knowledge gaps and involving risk assessors when determining national research agendas on antimicrobial resistance;
- to initiate a risk assessment on ESBL-producing bacteria.

Samenvatting

Sinds hun introductie in de jaren '40 van de vorige eeuw hebben antibiotica substantieel bijgedragen aan het verminderen van de ziektelast bij de mens. Het intensieve gebruik van deze middelen heeft echter ook een negatief effect; hierdoor vindt selectie en verspreiding van resistente bacteriën plaats, waardoor de initiële behandeling van sommige bacteriële infecties minder effectief is en de opties voor behandeling na het stellen van de diagnose beperkt zijn. In de dierhouderij worden antibiotica veelvuldig gebruikt waardoor ook hier de antibioticaresistentie toeneemt, zowel bij zoönotische als bij commensale bacteriën. Dit heeft geleid tot bezorgdheid over de risico's van overdracht van resistente zoönotische bacteriën (directe gevaren) en resistentiegenen (indirecte gevaren) van voedselproducerende dieren naar de mens en de mogelijke gevolgen daarvan voor de volksgezondheid en de gezondheidszorg. Dit rapport bevat een risicoprofiel, hetgeen bedoeld is om risicomanagers te informeren over de beschikbare kennis met betrekking tot dit potentiële gezondheidsrisico, als een eerste stap in het proces van risicomanagement. Drie gevaren (twee directe en één indirecte) dienen als voorbeeld in dit risicoprofiel, dit zijn:

- quinolone-resistente *Campylobacter jejuni*, (direct gevaar);
- veegerelateerde MRSA (v-MRSA), (direct gevaar);
- ESBL-producerende bacteriën, (indirect gevaar).

Hieronder volgt een samenvatting van relevante informatie betreffende hun risico's voor de humane gezondheid.

Quinolone-resistente *Campylobacter jejuni*

In de afgelopen decennia is de resistentie tegen quinolonen bij *Campylobacter*-isolaten van pluimvee en patiënten sterk gestegen en werd er een directe relatie met het gebruik van quinolonen in de pluimveesector aangetoond. Tijdgerelateerde, geografische en epidemiologische associaties leveren voldoende bewijs voor een oorzakelijk verband. Quinolone-resistente *Campylobacter* wordt, net als gevoelige *Campylobacter*, voornamelijk overgedragen door consumptie en bereiding van kippenvlees; dat andere transmissieroutes ook een rol spelen wordt in toenemende mate onderkend. Er zijn geen indicaties dat mensen in direct contact met voedselproducerende dieren of in de vleesverwerkende industrie een verhoogd risico lopen.
Campylobacter veroorzaakt in de eerste plaats een acute gastro-enteritis die gewoonlijk zelflimiterend is; er zijn geen indicaties dat er verschil is tussen de ziekte veroorzaakt door quinolone-resistente en -gevoelige stammen. Naar verwachting zal antibioticaresistentie het risico op chronische complicaties, zoals het Guillain-Barré-syndroom, reactieve artritis, het prikkelbare darm-syndroom en inflammatoire darmziekten, niet verhogen. Het gebruik van antibiotica is alleen geïndiceerd voor ernstig zieke of immuungecompromitteerde patiënten. Als gevolg van het hoge resistentieniveau van *Campylobacter* is het gebruik van quinolonen voor de initiële behandeling van gastro-enteritis uitgesloten in zowel de eerstelijnsgezondheidszorg als ziekenhuizen. Geschat wordt dat ongeveer 79.000 symptomatische *Campylobacter*-infecties per jaar plaatsvinden en ongeveer 50% van deze infecties (40.000 gevallen) wordt veroorzaakt door quinolone-resistente stammen. Er zijn geen aanwijzingen dat de ziektelast is toegenomen als gevolg van quinolone-resistentie en de kosten van de gezondheidszorg zijn dan ook vergelijkbaar met die voor gevoelige *Campylobacter*-infecties. De sociale gevolgen en risicoperceptie zijn niet onderzocht.
De opties voor de bestrijding van quinolone-resistente *Campylobacter* zijn dezelfde als die voor de bestrijding van gevoelige *Campylobacter* en omvatten zowel het terugdringen van de besmetting in de primaire productiesector als maatregelen met betrekking tot het slachtproces, zoals verbetering van de slachthygiëne en de combinatie van logistiek slachten met decontaminatie. Er zijn aanwijzingen dat het verbieden van het gebruik van fluoroquinolonen bij pluimvee het probleem niet oplost; in de VS heeft een dergelijk verbod niet geleid tot een verminderde fluoroquinolone-resistentie van patiëntenisolaten in de daaropvolgende jaren. Voor quinolone-resistente *Campylobacter* zijn verschillende 'risk assessments' beschikbaar. Ze tonen aan dat het gebruik van (fluoro)quinolonen bij voedselproducerende dieren bijdraagt aan resistentie bij de mens, maar de impact op de humane gezondheid lijkt relatief klein te zijn.

Veegerelateerde MRSA (v-MRSA)

V-MRSA werd voor het eerst ontdekt in 2005 en komt nu wijdverspreid voor in de Nederlandse intensieve veehouderij. De meerderheid van de stammen die zijn gevonden bij voedselproducerende dieren behoort tot het sequentie type 398. Op basis van tijdgerelateerde, geografische, epidemiologische en genetische associaties is er voldoende bewijs voor een oorzakelijk verband tussen humane klinische isolaten en isolaten van voedselproducerende dieren. Direct contact met levende dieren is de belangrijkste transmissieroute van voedselproducerende dieren naar de mens. De geïdentificeerde risicogroepen zijn mensen die wonen of werken op varkens- of kalverhouderijen, dierenartsen en werknemers in het slachthuis. Ongeveer 30% van de varkens- en kalverhouders in Nederland is drager van v-MRSA; het risico op dragerschap neemt toe naarmate de intensiteit en de duur van het contact met de dieren toeneemt. Overdracht van persoon naar persoon is

beperkt; dit verkleint het risico op secundaire verspreiding en limiteert uitbraken in het ziekenhuis. Er zijn geen aanwijzingen dat de aanwezigheid van v-MRSA op vlees een risico vormt voor de volksgezondheid of voor mensen werkzaam in de vleesverwerkende industrie.
V-MRSA veroorzaakt vooral huid- en wekedeleninfecties, maar kan ook invasieve infecties veroorzaken. In de afgelopen jaren werden jaarlijks ongeveer honderd v-MRSA-infecties gemeld; dit is ongeveer 10-15% van het totale aantal MRSA-infecties. De in de literatuur gerapporteerde klinische gevallen van v-MRSA betroffen mensen uit beroepsgroepen in direct contact met dieren, patiënten met andere onderliggende ziekten, oudere patiënten en patiënten die een operatie hadden ondergaan. V-MRSA is resistent tegen meerdere klassen antibiotica, waardoor de behandelopties beperkt zijn. De ziektelast en de -kosten (COI) van MRSA zijn niet gekwantificeerd, maar zijn waarschijnlijk toegenomen met de opkomst van de v-MRSA-infecties. Er zijn geen aanwijzingen dat de individuele ziektelast van v-MRSA verschilt van die van 'hospital-acquired' MRSA (HA-MRSA). MRSA-patiënten blijken psychische problemen te ervaren als gevolg van stigmatisering en isolatie. Risicoperceptie is niet onderzocht.
Momenteel zijn er, buiten de gezondheidszorg, geen specifieke bestrijdingsmaatregelen gericht op v-MRSA, met uitzondering van de algemene maatregelen in de veehouderij om het gebruik van antibiotica te verminderen. Tot de potentiële bestrijdingsmaatregelen in de primaire productiesector behoren aankoop van dieren van MRSA-negatieve bedrijven, restrictief gebruik van antibiotica en grondige reiniging en ontsmetting; het effect van deze maatregelen is echter nog niet gekwantificeerd. Opties voor preventieve maatregelen om blootstelling van professionals aan levende dieren en hun omgeving te verminderen zijn aanpassingen in dierlijke productiesystemen en het gebruik van persoonlijke beschermingsmiddelen. Persoonlijke beschermingsmiddelen zijn theoretisch effectief, maar als deze niet goed worden toegepast, kunnen deze het risico op besmetting juist verhogen.
Er zijn geen 'risk assessments' beschikbaar voor v-MRSA; voor beantwoording van het merendeel van de risico-gerichte vragen kan analytisch epidemiologisch onderzoek in combinatie met microbiële typering volstaan.

ESBL-producerende bacteriën

Breedspectrum bèta-lactamasen (Engels: extended-spectrum beta-lactamases, ESBLs) zijn enzymen die Gram-negatieve bacteriën resistent maken tegen bèta-lactam antibiotica en geremd worden door bèta-lactamase remmers. ESBL's zijn een belangrijke oorzaak van het mislukken van initiële cefalosporinebehandelingen van infecties veroorzaakt door ESBL-producerende bacteriën.
Sinds het begin van deze eeuw is het aantal ESBL-producerende bacteriën toegenomen onder zowel humane als veterinaire (met name pluimvee) isolaten. CTX-M is op dit moment het meest voorkomende ESBL-type onder mensen. ESBL-genen bevinden zich op plasmiden die kunnen worden overgedragen tussen bacteriesoorten. Deze plasmiden bevatten dikwijls ook andere antimicrobiële resistentiegenen, waardoor de bacteriën resistent zijn tegen meerdere klassen antibiotica. Op basis van tijdgerelateerde, genetische en epidemiologische associaties is er voldoende bewijs voor het bestaan van een relatie tussen plasmiden en de hierop gelegen ESBL-genen in humane klinische isolaten en isolaten van pluimvee; het bewijs is momenteel echter nog te beperkt om te concluderen dat er een causaal verband is. Recente gegevens suggereren dat zowel voedsel als direct contact een rol spelen bij de overdracht vanuit voedselproducerende dieren naar de mens. Uit pilot-onderzoek is gebleken dat fecaal dragerschap onder Nederlandse vleeskuikenhouders veelvuldig voorkomt (zes van de achttien onderzochte personen positief). Het is onduidelijk of de aanwezigheid van ESBL-producerende bacteriën op vlees een risico vormt voor de volksgezondheid of voor mensen werkzaam in de vleesverwerkende industrie.
In ziekenhuizen worden uitgebreide en bewerkelijke bestrijdingsmaatregelen genomen om de verspreiding van ESBL-producerende bacteriën te voorkomen. Behandelingsopties met alternatieve antibiotica zijn beperkt; deze zijn vaak duurder en kunnen ziekenhuisopname vereisen. In 2009 werden naar schatting 5400 urineweginfecties bij patiënten in de huisartsenpraktijk veroorzaakt door ESBL-producerende *E. coli*. Bovendien werden er in datzelfde jaar naar schatting 500 invasieve infecties veroorzaakt door ESBL-producerende bacteriën. De huidige kennis over risicofactoren voor dragerschap en infectie is beperkt. Geïdentificeerde risicofactoren zijn eerdere opname in gezondheidszorginstellingen, het gebruik van antibiotica, reizen naar hoogendemische landen en de aanwezigheid van ESBL-positieve gezinsleden. De ziektelast en -kosten zijn nog niet gekwantificeerd, maar zijn waarschijnlijk aanzienlijk en zullen toenemen. De sociale gevolgen en risicoperceptie zijn niet onderzocht.
Het besluit van de Nederlandse dierenartsen om te stoppen met het gebruik van cefalosporines bij pluimvee is de enige bestrijdingsmaatregel die momenteel wordt toegepast. Andere potentiële maatregelen in de primaire productie zijn restrictief gebruik van antibiotica en grondige reiniging en ontsmetting van stallen; het effect van deze maatregelen is echter nog onduidelijk. Een tijdelijke stop op het gebruik van cefalosporines bij pluimvee in Canada bleek effectief te zijn voor het verminderen van de prevalentie van ESBL-producerende bacteriën op pluimveevlees. Het uitvoeren van een

antimicrobiële 'risk assessment' (AMR-RA) zou van nut zijn bij de aanpak van de ESBL-problematiek.

Aanbevelingen

Op basis van de verzamelde informatie, worden de volgende aanbevelingen gedaan:

- monitoring van humane resistentie die geassocieerd wordt met het reservoir van voedselproducerende dieren door middel van verfijning van de bestaande humane surveillancesystemen;
- uitwerking van nationale onderzoeksagenda's om de geconstateerde kennislacunes met betrekking tot antimicrobiële resistentie in te vullen en het betrekken van risicobeoordelaars daarbij;
- uitvoering van een risicoschatting ('risk assessment') met betrekking tot ESBL-producerende bacteriën in voedselproducerende dieren.

Contents

1 Introduction

In food animal production, antimicrobial drugs are widely used and antimicrobial resistance is increasing. This has raised concerns about the transmission of antimicrobial resistance from food animals to humans and the consequences for health care and public health. The Ministry of Agriculture, Nature and Food quality (LNV, now EL&I) has commissioned the project ABRES-vet-med to develop a risk profile on this problem, which is presented in this report.
In this chapter the aim of the project and questions addressed in the risk profile are described in section 1.1. The risk profile is part of the risk management framework, which is described in section 1.2. Antimicrobial resistance terms used in this risk profile are listed in section 1.3.

1.1 Aim of the project ABRES-vet-med and questions addressed

The aim of the ABRES-vet-med project is *to develop a risk profile (this document) with respect to the transmission of antimicrobial resistance from food animals to humans and the consequences for health care and public health*. This risk profile is aimed to inform risk managers as a first step in the risk management process.

Based on the information presented in the risk profile, the following questions will be addressed:

1. What are the adverse effects of antimicrobial resistance to human health care and public health and what is the magnitude of these effects?
2. How strong is the evidence of an association between antimicrobial resistance in food animals and resistance in humans?
3. Through which routes does transmission occur?
4. To what extent does antimicrobial resistance in food animals contribute to resistance in humans?
5. What are the options for intervention and what is their presumed effectiveness?
6. Which future hazards can be anticipated?

This risk profile describes the state of the art in antimicrobial resistance at the interface of food animal production and human healthcare and public health. It describes the nature of the resistance problem, antimicrobial use, development of resistance, adverse health consequences for humans, and the magnitude of the impact on society (chapter 2), antimicrobial use, development of resistance, and presence of antimicrobial resistance in food and food animals and other reservoirs (chapter 3), associations and transmission routes (chapter 4), anticipated future hazards (chapter 5), possibilities for intervention with emphasis on prevention of transmission from food animals to humans (chapter 6), and risk assessment (chapter 7). The information primarily describes the Dutch situation; international data will be given when relevant.

Given the broad scope of this risk profile, the abovementioned topics will be illustrated by means of three relevant examples of antimicrobial resistance hazards (section 2.4). In the concluding chapter 8, the above mentioned questions will be answered for the three example hazards and recommendations will be given.

1.2 Risk management framework

Risk analysis provides risk managers and risk assessors with objective methods and transparent procedures to assess, manage and communicate public health risk issues like antimicrobial resistance. Several steps can be identified in the risk analysis process (Figure 1); the development of a risk profile is part of the preliminary risk management activities. The framework of the risk management process for antimicrobial resistance is specified below and is based on (Codex, 2007) and (Codex, 2009).

1. Preliminary risk management activities

a. *Identification of risk managers*
The Dutch Ministry of EL&I is responsible for policy making with respect to reduction of the transmission of antimicrobial resistance from food animals to the human population. The ministry is therefore identified as the main risk manager.

b. *Identification of a public health issue*
The health hazard of antimicrobial resistance is the development of resistance in a pathogenic bacterium, as well as the development of a resistance determinant that may be passed to other bacteria that are pathogenic (Vose et al., 2001b). Use of antimicrobials in food animals may result in reduced susceptibility of human pathogenic bacteria to one or several antimicrobial drugs. Humans exposed to these pathogens may fall ill and, as a consequence of the reduced therapeutic value of the antimicrobials used, may suffer from prolonged illness and/or a higher risk of death.

c. *Risk profile*
The risk profile (this document) reviews relevant information with respect to spread of antimicrobial resistance from food animals to the human population and the consequences for human health. The information is reviewed in a structured way and follows a risk based approach. The objectives of the risk profile are to support further risk management activities by informing the risk manager on the context of the problem, data gaps, the feasibility of a risk assessment, and potential management options and priorities. Based on the outcomes of a risk profile, the risk manager may decide the following actions: no action, initiation of a risk assessment or, in case of an urgent public health concern, immediate (provisional) action (Codex, 2007).

d. *Risk assessment policy*
A risk assessment policy should be established and documented by the risk manager in close collaboration with the risk assessors before carrying out a risk assessment. The risk assessment policy aims to protect the scientific integrity of the assessment and offers guidance with respect to possible sources of subjectivity e.g. uncertainties, choice of data sources, data gaps, value judgments, policy choices, etcetera (Codex, 2007).

e. *Commissioning of a risk assessment and consideration of the process and the results*
Risk assessments are commissioned by the risk manager and carried out by the risk assessors. Based on the risk profile a choice is made for an 'antimicrobial-resistant bacterium - antimicrobial use - specified transmission route' combination to be assessed and a provisional list of risk management options to be evaluated (Codex, 2009). It is also possible to commission a comparative exposure assessment in which the attribution of various pathways can be compared. The results of the assessment should be presented by the risk assessors in a clear and transparent way in order to be properly understood by the risk manager. Special attention should therefore be given to the strengths and limitations of the assessment: assumptions, uncertainty, variability in data and data sources and their influence on the outcomes (Codex, 2007).

2. Identification and selection of risk management options

The risk manager has to consider all possible risk management options identified in the risk profile and select a suitable option or combination of options for practical implementation taking into account all evaluation information obtained from the risk profile and risk assessment. The selection of options should be based on their ability to reduce the risk posed by antimicrobial resistance transmitted from food animals to the human population to an appropriate level, possible effects on animal health, advantages/ disadvantages, and practical feasibility (Codex, 2007; Codex, 2009).

3. Implementation of risk management options

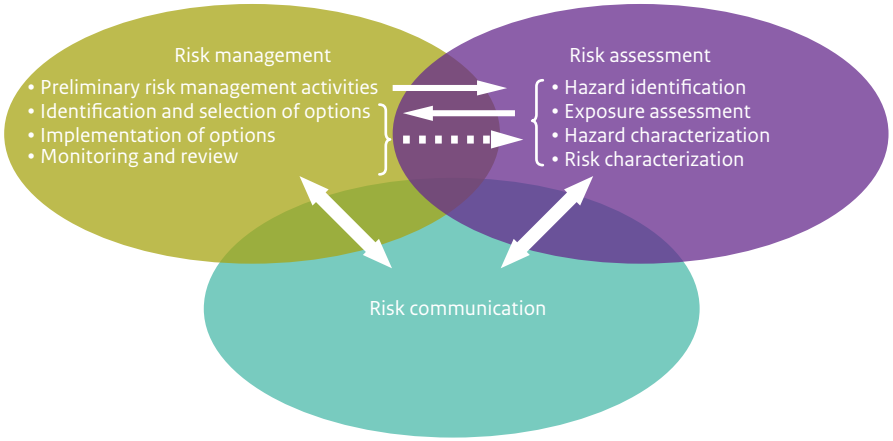
Implementation of risk management options include the actual realization of the options and verification that they are implemented as intended. Several stakeholder groups may be involved (authorities, farmers, veterinarians, pharmaceutical industry, food industry, health care, consumers, patients, etcetera). To ensure transparency, decisions on management risk options should be communicated by the risk managers to all stakeholders involved. Minimum measures that should

be implemented are prudent use guidelines and general on-farm and food hygiene principles (Codex, 2007).

4. Monitoring and review

Monitoring and review activities are essential parts of the risk management process. Monitoring is the continuous gathering, analysing and interpreting of data related to the use of antimicrobials, antimicrobial resistance development in selected bacteria, surveillance of clinical disease related to antimicrobial-resistant agents, etcetera. (Codex, 2009). This is an iterative process, as monitoring is essential before, during and after implementation of the risk management options, to establish a baseline, to compare the effectiveness of new risk management activities and to support the risk manager on decisions of further steps to be taken. Review activities measure the effectiveness, appropriateness and implementation of the selected risk management options and may lead to a change in risk management activities. Risk management options should be reviewed regularly or whenever new relevant information becomes available (Codex, 2007).

Figure 1 Risk analysis framework (FAO/WHO, 1997).



1.3 Glossary

Acquired resistance:	the resistance that is acquired either by mutation or by the uptake of exogenous genes by horizontal transfer from other bacterial strains (EFSA, 2008b).
Activated sludge:	a semi-liquid mass of aerated precipitated sewage containing micro-organisms which is added to untreated sewage to reduce organic pollution. Sludge of treated sewage can be used as fertilizer.
Antibiotic:	see antimicrobial (drug).
Antimicrobial resistance risk assessment (AMR-RA):	a scientific tool to qualitatively or quantitatively evaluate the health risk resulting from exposure to resistant bacteria or resistance genes (Codex, 2009).
Antimicrobial resistance:	the capacity of bacteria to survive exposure to a defined concentration of an antimicrobial (EFSA, 2008b).
Antimicrobial (drug):	any substance of natural, semi-synthetic, or synthetic origin that kills or inhibits the growth of micro-organisms by interacting with a specific target at in vivo concentrations (FAO/OIE/WHO, 2008), also referred to as antibiotic. In this risk profile, the term antimicrobial/antibiotic will be limited to antibacterial drugs that are used for therapeutic or preventive use in food animals and/or humans, unless otherwise stated.
Aquaculture:	the cultivation of aquatic organisms (as fish or shellfish) especially for food.
Bactericidal antibiotics:	antibiotics that kill bacteria (Wikipedia)
Bacteriophage:	a virus that lyses bacteria (Dorland's medical).
Bacteriostatic antibiotics:	antibiotics that limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism (Wikipedia)
Carrier:	an individual who harbors the specific organisms of a disease without manifest symptoms and is capable of transmitting the infection (Dorland's medical).

Clinical resistance:	infections having a low probability of clinically responding to treatment, even if maximum doses of a given antimicrobial are administered (EUCAST, 2000 and Acar and Röstel, 2003 in EFSA, 2008b).
Colonization:	the foundation and growth of a new group of microorganisms on a host (Dorland's medical)
Commensal bacteria:	bacteria that live on or within another organism without causing injury to their host (Dorland's medical).
Conjugation:	a form of sexual reproduction in which nuclear material is exchanged during the temporary fusion of two cells (conjugants), (Dorland's medical).
Co-resistance:	two or more different resistance genes that are physically linked e.g because they are contained in larger genetic elements such as integrons, transposons or plasmids (EFSA, 2008b).
Co-selection:	concurrent selection of genetic traits that are linked, e.g. virulence and resistance genes.
Cost of illness:	framework to calculate the cost of illness for society (Kemmeren et al., 2006).
Cross contamination (of food):	the transfer of micro-organisms from one food to another.
Cross resistance:	resistance to either several antimicrobials within one class due to a similar mode of action and/or a similar target, or resistance to antimicrobials in unrelated classes due to less specific mechanisms of resistance such as efflux pumps or overlap in bacterial targets (EFSA, 2008b).
Defined Animal Daily Dose (ADD):	the assumed maintenance dose per day for a drug for its main indication in a specific animal species.
Defined Daily Doses (DDD):	the assumed average maintenance dose per day for a drug used for its main indication in adults (WHO, 2009).
Direct (health) hazard:	hazard that directly affects human health (e.g. resistant zoonotic pathogens).
Direct health care costs (DHC):	all costs that are directly connected to prevention, diagnostics, therapy, revalidation and the care of patients, (Kemmeren et al., 2006).
Direct non-health care costs (DNHC):	costs that patients make due to disease (e.g. time and travel costs), (Kemmeren et al., 2006).
Disability Adjusted Life Year (DALY):	one lost year of healthy life (WHO, measure of disease burden).
(Disease) susceptibility:	diminished immunity to a disease, especially an infection (Dorland's medical).
Effluent:	the outflow of water from a (waste water) treatment plant.
Empiric treatment:	the initial treatment of an infection prior to the definitive diagnosis on the causative agent and its antimicrobial resistance (Wikipedia).
Exposure assessment:	second step in AMR-RA; identifies the pathways of exposure and aims to estimate the frequency and amount of the (antimicrobial resistance) hazard to which humans are exposed (Codex, 2007).
Fomite:	an object that is not in itself harmful, but is able to harbor pathogenic microorganisms and thus may serve as an agent of transmission of an infection (Dorland's medical)
Fresh produce:	farm-produced goods, especially vegetables and fruit, that are in the same state in stores as when they were harvested.
Gram-negative:	losing the stain or decolorized by alcohol in Gram's method of staining, a primary characteristic of bacteria having a cell wall composed of a thin layer of peptidoglycan covered by an outer membrane of lipoprotein and lipopolysaccharide (Dorland's medical).
Gram-positive:	retaining the stain or resisting decolorization by alcohol in Gram's method of staining, a primary characteristic of bacteria whose cell wall is composed of a thick layer of peptidoglycan with attached teichoic acids (Dorland's medical).
Growth promotor:	any medicine that destroys or inhibits bacteria and is administered at a low, subtherapeutic dose (website fao).
Habitat:	the area or environment where an organism or ecological community normally lives or occurs.
Hazard characterization:	third step in AMR-RA; aims to determine the probability of disease as a consequense of exposure to the (antimicrobial resistance) hazard (Codex, 2007).
Hazard identification:	First step in AMR-RA; aims to identify the (antimicrobial resistance) hazard (Codex, 2007).

Hazard:	a biological, chemical or physical agent with the potential to cause an adverse health effect (Codex, 2007).
Horizontal gene transfer:	exchange of genetic material between two microorganisms; no new microorganism is created (http://www.tufts.edu/med/apua/Miscellaneous/Glossary.html).
Immunocompromised:	having the immune response attenuated (Dorland's medical).
Indirect (health) hazard:	hazard that indirectly affects human health (e.g. resistance genes).
Indirect health care costs (IHC):	the future savings on health care that arise as a secondary consequence of the illness or treatment, (Kemmeren et al., 2006).
Indirect non-health care costs (INHC):	the value of production lost to society due to disease, (Kemmeren et al., 2006).
Infection:	invasion and multiplication of microorganisms or parasites in body tissues; it may be clinically inapparent (subclinical infection) or remain localized (Dorland's medical).
Integrons:	a two component gene capture and dissemination system, initially discovered in relation to antibiotic resistance, and which is found in plasmids, chromosomes and transposons (Wikipedia).
LOS:	length of stay, the number of days a patient stays in a hospital or other health care facility
Microaerophilic:	requiring oxygen for growth but at lower concentration than is present in the atmosphere (Dorland's medical).
Microbiological resistance:	toleration of higher concentrations of an antimicrobial than phenotypically related bacteria of the original or 'wild type' strain (Acar and Röstel, 2003 in EFSA, 2008b).
Minimum inhibitory concentration (MIC):	the lowest concentration of an antimicrobial that will inhibit visible growth of the bacterium after overnight incubation (Wikipedia).
Mobile genetic elements:	segments of DNA that can move around within the genome, e.g. plasmids and transposons (Wikipedia).
Multidrug-resistance (MDR):	resistance to multiple classes of antimicrobial drugs . Note: the number of classes is not standardized (EFSA, 2008b).
Mutation:	changes in the DNA sequence of a cell's genome (Wikipedia).
Non-wild type:	microorganism with acquired or mutational resistance to a specified antimicrobial drug (Kahlmeter et al., 2003).
Nosocomial infection:	an infection not present or incubating prior to admittance to a hospital, but occurring a few days after admittance; the term is usually used to refer to patient disease, but hospital personnel may also acquire nosocomial infection (Dorland's medical)
Opportunistic pathogen:	a microorganism that does not ordinarily cause disease but that, under certain circumstances (e.g., impaired immune responses resulting from other disease or drug treatment), becomes pathogenic (Dorland's medical).
Outpatients:	a patient who comes to the hospital, clinic, or dispensary for diagnosis and/or treatment but does not occupy a bed (Dorland's medical)
Pan-drug resistance:	resistance to all available classes of antimicrobial drugs
Pathogenicity island:	part of the genome that contains one or more virulence factors and can be transferred by horizontal gene transfer.
Phagetherapy:	the therapeutic use of bacteriophages to treat bacterial infections (Wikipedia).
Plasmid:	an extrachromosomal self-replicating structure found in bacterial cells that carries genes for a variety of functions not essential for cell growth (Dorland's medical).
Public health risk:	a function of the probability of an adverse health effect and the severity of that effect in a human population, as a consequence of a hazard (Codex, 2007).
Reservoir (host):	an alternate or passive host or carrier that harbors pathogenic organisms or parasites, without injury to itself, and serves as a source from which other individuals can be infected (Dorland's medical).
Risk analysis:	a process consisting of three components: risk assessment, risk management and risk communication (Codex, 2007).
Risk assessment:	a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization (Codex, 2007).
Risk characterization:	fourth step in AMR-RA; integrates the results of the preceding steps and aims to generate an overall estimate of the health risk (Codex, 2007).

Risk communication:	the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (Codex, 2007).
Risk management:	the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of human beings and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options (Codex, 2007).
Risk perception:	the subjective judgment that people make about the characteristics and severity of a risk (Wikipedia)
Risk profile:	the description of the public health issue and its context (Codex, 2007).
Scheduled processing:	to separate positive and negative flocks for slaughter, followed by freezing or chemical decontamination of the meat of the positive flocks (Wagenaar et al., 2006).
Selective pressure:	the intensity of selection acting on a population of bacteria e.g. as a result of antimicrobial use. Its effectiveness is measured in terms of survival and reproduction, and consequently in change in the frequency of alleles in a population (adapted from FAO: http://www.fao.org/docrep/003/x3910e/X3910E22.htm).
SOS response:	the synthesis of a whole set of DNA repair, recombination and replication proteins in bacteria containing severely damaged DNA (Biotechnology glossary FAO website: http://www.fao.org/docrep/003/x3910e/X3910E22.htm).
Transduction:	a method of genetic recombination in bacteria, in which DNA from a lysed bacterium is transferred to another bacterium by bacteriophage, thereby changing the genetic constitution of the second organism (Dorland's medical medical).
Transformation:	the exchange of genetic material between strains of bacteria by the transfer of a fragment of naked DNA from a donor cell to a recipient cell, followed by recombination in the recipient chromosome (Dorland's medical medical).
Transposon:	a small mobile genetic (DNA) element that can move around within the genome or to other genomes within the same cell, usually by copying itself to a second site but sometimes by splicing itself out of its original site and inserting in a new location (Dorland's medical medical).
Vertical gene transfer:	the transfer of genes from a bacterium to its offspring.
Virulence:	the relative capacity of a bacterium to cause damage in a host (Casadevall and Pirofski, 1999)
Waste water treatment plant:	a facility where waste water is processed to improve its chemical and biological composition to a point that it can safely be released to the environment.
Wild type:	microorganism without acquired or mutational resistance to a specified antimicrobial drug (Kahlmeter et al., 2003).
Years Lost due to Disability (YLD):	total number of years that patients have spent with disease in a human population (Havelaar, 2007).
Years of Life Lost (YLL):	the total number of years lost due to premature death in a human population (Havelaar, 2007).
Zoonosis:	disease transmitted between vertebrate animals and man under natural conditions (Van der Giessen et al., 2010)

2 Description of the micro-biological hazards

This chapter focusses on the specific microbiological aspects of the health hazards studied in this risk profile. These hazards were identified in section 1.2 as the development of resistant bacteria and their resistance genes originating in food animal production that directly or indirectly may cause adverse health effects in humans. Section 2.1 provides background information on antimicrobials and antimicrobial resistance. Sections 2.2-2.4 describe the direct and indirect health hazards of antimicrobial resistance and the selection of three agents of concern that are used as examples throughout this risk profile.

2.1 Nature of the problem

Definitions of antimicrobials and antimicrobial resistance

Antimicrobials or antimicrobial agents are defined as: 'Any substance of natural, semi-synthetic, or synthetic origin that kills or inhibits the growth of micro-organisms by interacting with a specific target at in vivo concentrations' (FAO/WHO/OIE, 2008).

This very broad definition includes antibacterial, antiviral, antifungal, and antiparasitic agents. In this risk profile, the term antimicrobial is limited to antibacterial drugs (also named antibiotics) that are used for therapeutic or prophylactic use in food animals and/or humans to treat or prevent bacterial infections. Antimicrobials disturb vital processes of bacteria resulting in either growth inhibition (bacteriostatic antibiotics) or killing the bacteria (bacteriocidal antibiotics).

Antimicrobial resistance is generally defined as: 'The capacity of bacteria to survive exposure to a defined concentration of an antimicrobial' (EFSA, 2008b).

When bacterial populations are exposed to antimicrobials (selective pressure), resistant bacteria will have a selective advantage over the susceptible ones and the resistant fraction in the population will increase. As a consequence of resistance, antimicrobial drugs become less effective or ineffective for treatment of bacterial infections.

Origin and development of antimicrobials

The majority of antimicrobials that are used for therapeutic or prophylactic use in food animals and humans have an environmental origin. The production of antimicrobials by fungi and bacteria is a natural phenomenon in environmental microbial populations, e.g. soil, where many microorganisms have to compete for suitable niches (Martínez, 2008). For example, penicillin, the first antimicrobial that was discovered and used therapeutically, is produced by the fungus *Penicillium chrysogenum*. Most antimicrobials currently used for therapeutic goals are produced semi-synthetically or fully synthetically. Nowadays there are hundreds of therapeutic antimicrobials, which are subdivided into several classes and subclasses based on their chemical structure (Appendix).

Mechanisms of antibiotic action

There are five main categories of the mechanisms of antibiotic action (Tenover, 2006):

- interference with cell wall synthesis
- inhibition of protein synthesis
- interference with DNA/RNA synthesis
- inhibition of metabolic pathways
- disruption of bacterial membrane structures

Emergence of resistance

Antimicrobial resistance mechanisms arise through mutation of bacterial genes. In bacteria, mutations occur spontaneously with a mutation rate of approximately 10-10 per base pair and per generation (Drake, 1999). When bacteria are stressed, e.g. when exposed to antimicrobials, mutation rates increase as a result of an activated bacterial stress response system, the SOS response. This may result in resistant mutants that are better adapted to the worsened conditions, which potentially speeds up antimicrobial resistance (Galhardo et al., 2007).

Mechanisms of antimicrobial resistance

Four main mechanisms of antimicrobial resistance can be distinguished (EFSA, 2008b; Tenover, 2006):

- target alteration, e.g. production of cell walls that have no or altered binding sites
- production of enzymes that inactivate or degrade the antimicrobial drug
- permeability changes, by either limited access by altered porins (transmembrane proteins) or by efflux pumps that pump out the antimicrobial drug
- alternative metabolic pathways

Measurement of resistance

Antimicrobial resistance of a bacterium is generally determined *in vitro* and based on its survival to a defined concentration of an antimicrobial. Resistance is usually expressed as the minimum inhibitory concentration (MIC), i.e. the lowest concentration of an antimicrobial that will inhibit visible growth of the bacterium. Threshold values or breakpoints are used to define whether a bacterium is susceptible or resistant to an antimicrobial; these thresholds depend on the objective of the investigation (see resistance terminology below).

Resistance terminology

Depending on the objective of the investigation, there are two definitions of resistance: clinical and microbiological resistance. Clinical resistance means that the MIC of an antimicrobial for the bacterium is associated with a high likelihood of therapeutic failure of treatment with this drug (EFSA, 2008b). Microbiological resistance means that the MIC of the antimicrobial is higher than expected for wild type strains (EFSA, 2008b). In this report, resistance means microbiological resistance unless otherwise stated.

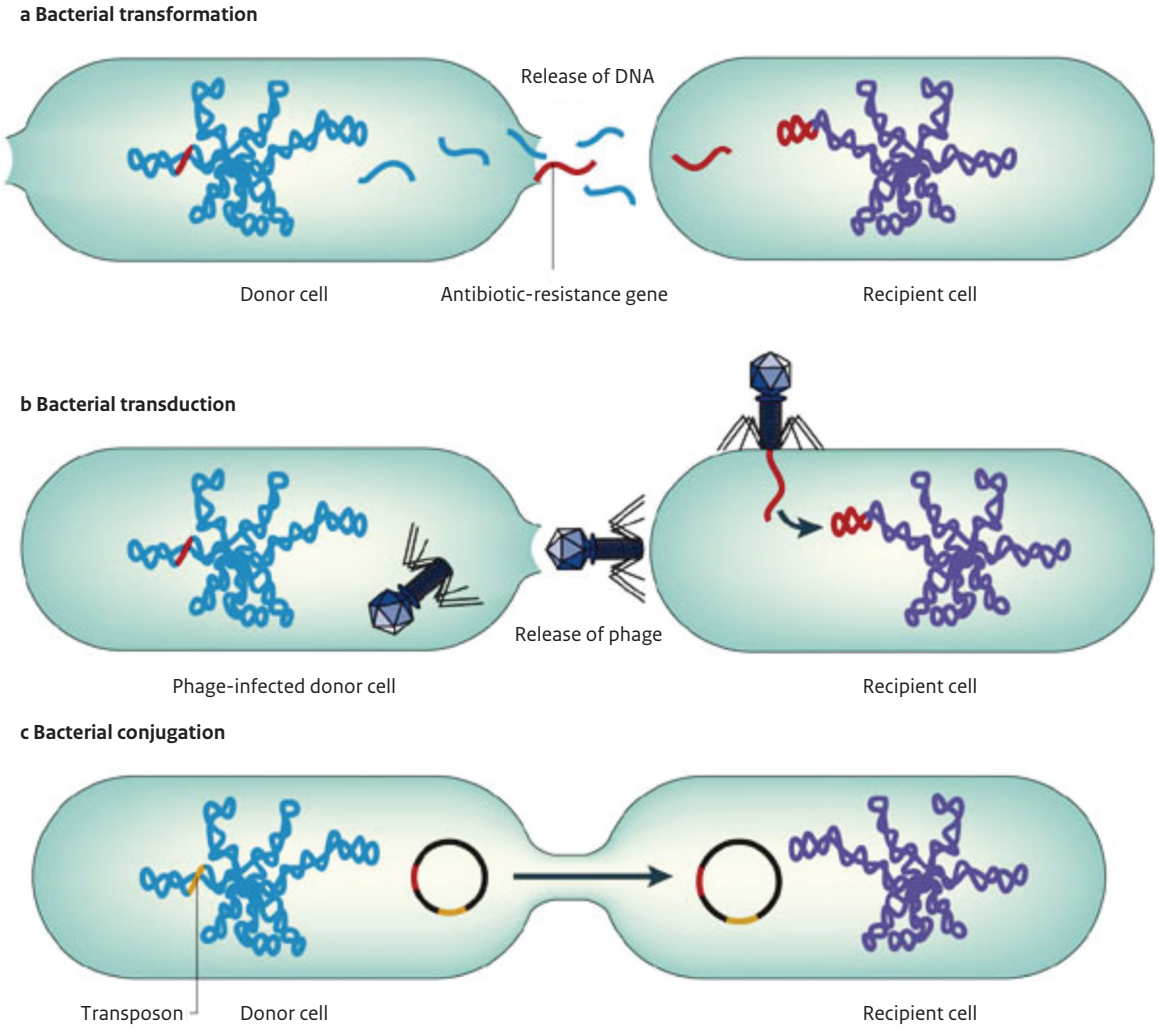
There are several definitions of resistance in use that refer to the origin of the resistance, e.g. acquired resistance (resistance acquired by mutation or horizontal transfer), anthropogenic resistance (resistance as the result of human activities) and natural resistance (or intrinsic or autochthonous resistance), which refers to resistance that is present in wild type strains in nature that makes these bacteria insensitive to the antimicrobial.

Bacteria that are resistant to one antimicrobial of a certain class, usually are resistant to all other antimicrobials in the same class due to a similar mode of action and/or a similar target. This is referred to as cross-resistance. Cross resistance may also occur in unrelated classes e.g. due to less specific mechanisms of resistance such as enhanced efflux pumps or overlap in bacterial targets. Co-resistance means that resistance genes are physically linked together e.g. when they are situated on the same plasmid. As a consequence selection for one resistance gene will also select for the resistance genes that are linked to it. Finally, multidrug resistance (MDR) means that a bacterial strain is resistant to different classes of antimicrobials.

Spread of antimicrobial resistance

Spread of antimicrobial resistance from animals to humans may take place by transmission of antimicrobial-resistant bacteria or by transfer of antimicrobial resistance genes. Antimicrobial-resistant bacteria that are transmitted from animals to humans and potentially cause disease in humans, i.e. resistant zoonotic bacteria, pose a direct hazard (see also 2.2). Transmission of antimicrobial-resistant bacteria may occur via direct contact, food or environmental routes, which are discussed in more detail

Figure 2 Horizontal gene transfer between bacteria (from: Furuya and Lowy, 2006)



in chapter 4. Bacteria of food-producing animals that do not cause disease in humans, but potentially transfer their resistance genes to pathogenic bacteria of humans, i.e. horizontal gene transfer, pose an indirect hazard (see also section 2.3). Mechanisms of horizontal gene transfer are described below.

Mechanisms of gene transfer

Transfer of resistance genes may occur through vertical gene transfer or horizontal gene transfer. Vertical gene transfer is the transfer of genes from a bacterium to its offspring. Horizontal gene transfer is the exchange of genetic material from a donor bacterium to a recipient bacterium that is not its offspring. There are three mechanisms for horizontal gene transfer: conjugation, transduction and transformation (see also Figure 2). With these processes, genetic material is moved between bacteria by either a temporary linkage between a donor and recipient bacterium (conjugation), by bacteriophages (transduction) or by uptake of free DNA (transformation). Conjugation is the most commonly reported antimicrobial gene transfer mechanism and may occur between bacteria of different species or genera (EFSA, 2008b). During conjugation, mobile genetic elements e.g. plasmids or transposons, can be transferred. Integrons are gene capture systems that can be found in plasmids, chromosomes and transposons and play an important role in the dissemination of genetically linked antimicrobial resistance by the capture, mobilization, and expression of resistance genes (Kovalevskaya, 2002). Published gene transfer rates during conjugation were found to vary widely (Hunter et al., 2008). Besides increased mutation rates, the SOS response also promotes horizontal gene transfer (Beaber et al., 2004).

Persistence of antimicrobial resistance in bacterial populations

Acquisition of antimicrobial resistance may result in a reduced biological fitness of the bacterium (Andersson, 2003). As a result, resistant bacteria may be outcompeted by susceptible bacteria in environments where antibiotic selection pressure is absent. However, several studies have shown that after removal of the selective pressure, i.e. after withdrawal of the use of the antimicrobial, antimicrobial resistance may persist for many years though often at a lower level (Sørum et al., 2006). This can be explained by several mechanisms (Andersson, 2003) and (Zhang et al., 2006):

- compensatory mutations
- no-cost or low cost of resistance
- enhanced fitness (Luo et al., 2005)
- genetic linkage to other resistances (i.e. co-resistance)
- gene silencing (Enne et al., 2006)
- plasmid addiction systems (Woodford et al., 2009)
- besides these mechanisms, the epidemiological features (e.g. transmissibility, survival in the environment) are also of importance for persistence of antimicrobial-resistant bacteria (Garcia-Migura et al., 2007)

Virulence and antimicrobial resistance

The virulence of a bacterium is its relative capacity to cause damage in a host (Casadevall and Pirofski, 1999). Virulence factors are factors related to invasiveness, infectiousness or toxigenicity of the bacterium. Virulence is often encoded by several genes, which are frequently found on mobile genetic elements or pathogenicity islands that can be transferred by horizontal gene transfer. Genes determining virulence and antimicrobial resistance genes can be genetically linked in a bacterium, e.g. on mobile genetic elements. As a consequence co-selection may take place, i.e. selection pressure induced by antimicrobials may select for more virulent bacteria. Fortunately, examples of enhanced virulence of antimicrobial-resistant bacteria, e.g. increased disease manifestation and colonization in community acquired MRSA (Diep and Otto, 2008), are rare. In absence of antimicrobial drug use, antimicrobial resistance may still influence the virulence of a bacterium. For example, genes that have a function in virulence as well as antimicrobial resistance have been described (Quinn et al., 2007). The complex relationship between virulence, transmissibility, and antimicrobial resistance is discussed at length by (Martínez and Baquero, 2002).

2.2 Direct hazards of antimicrobial resistance

Antimicrobial-resistant bacteria that are transmitted from vertebrate animals to humans under natural conditions and potentially cause disease in humans (zoonoses), pose a direct hazard for human health. Antimicrobial resistance may reduce the efficacy of initial empirical treatment of the zoonosis and limit the choice of treatment after diagnosis. Resistant strains of (foodborne) zoonotic bacteria may cause a longer duration of illness, more invasive illness, higher mortality, and increased risk of hospitalization than susceptible strains (Mølbak, 2005).

Antimicrobial resistance in zoonotic bacteria

In the Netherlands, several bacterial zoonoses may occur and varying levels of antimicrobial resistance have been found in the causative agents (overview, see Table 1). The majority of these zoonoses are linked to reservoirs in animal husbandry; the main exposure sources are food products of animal origin.
Surveillance programs on the prevalence of antimicrobial resistance are performed for the zoonotic agents *Salmonella* spp., *Campylobacter* spp. and STEC O157 (MARAN-2008) and an extensive research program for livestock-associated MRSA has been carried out in the Netherlands (Wagenaar and Van de Giessen, 2009). It is increasingly reported that part of the human urinary tract infections caused by commensal *E. coli* may also originate from the food animal reservoir (Jakobsen et al., 2010; Johnson et al., 2007), but conclusive evidence is lacking.

2.3 Indirect hazards of antimicrobial resistance

Use of antimicrobials in food animals will promote the development of antimicrobial resistance in both pathogenic and commensal bacteria (Varga et al., 2009). Resistance genes carried on mobile genetic elements may be transferred to the human flora by horizontal gene transfer during transit or colonization of the human body (EFSA, 2008b; Hunter et al., 2008). Consequently the human flora may become resistant, including bacteria that are potentially harmful to humans, e.g. nosocomial pathogens (Donskey, 2004). These resistance genes thereby pose an indirect hazard.

Transfer of antimicrobial resistance genes from animals to humans

Horizontal gene transfer has been responsible for the dissemination of numerous antimicrobial-resistance genes among various bacterial species (Barlow, 2009). In particular the gastrointestinal tract is an important hot-spot for horizontal inter- and intra-species gene transfer. Major residents of the mammalian gastrointestinal tract, e.g. *Enterococcus* spp. and *Escherichia coli*, possess a wide spectrum of mobile genetic elements and have been shown to be potent donors and receivers of antimicrobial resistance genes, e.g. glycopeptide resistance in *Enterococcus* spp. and beta-lactam resistance in *E. coli*.

Table 1 Overview of the main bacterial zoonoses in the Netherlands (Valkenburgh S, 2007; website ziekdoordier) and their antimicrobial resistance

Zoonose	Agent	Animal reservoirs	Average number of human cases/yr	Antimicrobial resistance
Botulism	*Clostridium botulinum*	Mammals, birds, fish, environment	Rare; intoxication	---—
Brucellosis	*Brucella* spp.	Cattle, goats, sheep, dogs	Rare; travel or import related	Reduced susceptibility to rifampicin and sulfamethoxazole-trimethoprim found, susceptible to most antibiotics for treatment [3]
Campylobacteriosis	*Campylobacter* spp.	Mammals and birds; especially poultry	80,000 symptomatic cases in the general population, 6700 lab confirmed cases [1]	Increasing resistance to ciprofloxacin + multidrug resistance. Erythromycin resistance in imported products [4]
Cat-scratch disease	*Bartonella henselae*	Cats	300-1000 estimated cases	---—
Leptospirosis	*Leptospira* spp.	Rats, mice, cattle [5]	30 reported cases	—
Listeriosis	*Listeria monocytogenes*	Cattle, goats, sheep (note: common in environment)	69 reported cases	Most isolates susceptible, slow increase of resistance [5]. In NL resistance to sulfamethoxazole found.
Lyme disease	*Borrelia burgdorferi*	Tick borne; deer rodents, cattle, sheep, dogs	17,000 cases with erythema migrans in 2005	No scientific evidence for acquired antimicrobial resistance *6*
LA-MRSA	*Staphylococcus aureus*	Cattle, pig, poultry, horses	90 symptomatic cases	Resistant to ciprofloxacin, beta-lactams, tetracycline, erythromycin, lincomycin, gentamicin, kanamycin, doxycycline, tobramycin, and clindamycin [7, 8]
Psittacosis	*Chlamydophila psittaci*	Birds, incl. poultry	50-70 cases year (underestimate)	--
Q-fever	*Coxiella burnetti*	Cattle, goats, sheep	15 cases, more than 2000 cases in the 2009 epidemic	--
Salmonellosis	*Salmonella* spp.	Mammals, birds, reptiles, amphibians; especially poultry, pigs	30,000-45,000 symptomatic cases in the general population, 2700 lab confirmed cases [1]	Increasing levels + multidrug resistance. Quinolone resistance related to travel and imported products [4]
STEC*	*Escherichia coli*	Cattle, sheep	1250 cases of gastroenteritis, 180 at GP, 20 HUS [9]	Rare [4]
Tuberculosis	*Mycobacterium bovis*	Cattle [5]	Rare; 11 reported cases in 2005 [2]	Multi and extensive drug resistance [2]

-- = no information found on acquired resistance; * Shiga toxin-producing *Escherichia coli*; # Livestock-associated methicillin-resistant *Staphylococcus aureus*; [5] official free status in animal husbandry; [1] (Van Pelt et al., 2008a); [2] (Erkens, 2008), [3] (Turkmani et al., 2006); [4] (MARAN-2008); [5] (Conter et al., 2009); [6] (Hunfeld and Brade, 2006); [7] (Van Loo et al., 2007); [8] (Van Duijkeren et al., 2008); [9] (Havelaar et al., 2009)

Transfer of antimicrobial resistance genes from animals to humans (i.e. from donor bacteria of animal origin and recipient bacteria from human origin) has been shown in vitro and in vivo (De Niederhäusern et al., 2004; Lester et al., 2006). The association of antimicrobial resistance between (food-producing) animals and humans is more complex for indirect hazards than direct hazards, but the ultimate impact on health may be many times greater (Mevius, 2009), see also section 4.1.

2.4 Example agents

Based on urgency, data availability and host-pathogen characteristics, two direct hazards and one indirect hazard were selected to serve as examples throughout this risk profile. The selected agents are:

- quinolone-resistant *Campylobacter jejuni* (direct hazard)
- LA-MRSA (direct hazard)
- ESBL-producing bacteria (indirect hazards)

For the ESBL-producing bacteria and genes, focus will be on ESBL-producing *Escherichia coli* and *Salmonella* spp. in animal husbandry and ESBL-producing *E. coli* and *Klebsiella* spp. in human health. The general characteristics of the two bacteria and the beta-lactam inactivating enzymes are briefly described below.

Quinolone-resistant *Campylobacter jejuni*

Campylobacter jejuni is a Gram-negative, microaerophilic, motile, spiral-shaped bacterium. Its natural habitat is the intestinal tract of mammals and birds. Campylobacteriosis is the most common zoonosis in the Netherlands. The last decade, a strong increase in quinolone resistance in *Campylobacter* isolates of poultry and of human infections was observed and is found to be directly related to the use of the fluoroquinolone enrofloxacin in poultry (Endtz et al., 1991).

Livestock-associated MRSA (LA-MRSA)

Staphylococcus aureus is a Gram-positive, facultative anaerobe, non-motile, coc-shaped bacterium that appears as grape-like clusters under the microscope. Its natural habitat is the skin, nasal cavity and orofarynx of mammals and birds. Meticillin-resistant *S. aureus* (MRSA) are resistant to all beta-lactam antibiotics and to various other classes of antimicrobial drugs, which makes infections with this bacterium more and more difficult to treat. Livestock-associated MRSA (LA-MRSA) was discovered in 2005 in the Netherlands and is now widespread among pigs and veal calves (Wagenaar and Van de Giessen, 2009).

Extended-spectrum beta-lactamase (ESBL) producing bacteria

Beta-lactamases are enzymes produced by Gram-negative bacteria that cleave the amide bond in the beta-lactam ring, rendering the bacteria resistant to beta-lactam antimicrobials. Extended-spectrum beta-lactamases (ESBLs) are beta-lactamases capable of inactivating penicillins, cephalosporins and aztreonam and are inhibited by beta-lactamase inhibitors (Paterson and Bonomo, 2005). Several types of ESBLs can be distinguished, e.g. SHV-, TEM-, and CTX-M beta-lactamases. The CTX-M type is currently the most predominant type and comprises more than 80 enzymes (http://www.lahey.org/studies/). ESBL-encoding genes are usually located on plasmids that often also carry other antimicrobial resistance genes, rendering the bacteria resistant to multiple antimicrobials and making co-selection likely. Both clonal spread and transfer of mobile genetic elements between several Gram-negative bacterial species may occur (Cantón and Coque, 2006). The number of ESBL-producing Gram-negative bacteria is rising considerably both in humans and animals (EARRS, 2009; MARAN-2008).

The example ESBL-producing bacteria in this risk profile (*E. coli*, *Salmonella* spp. and *Klebsiella* spp.) are Gram-negative, facultative anaerobe, rod-shaped bacteria. Their natural habitat is the intestinal tract of animals and humans, but they may also survive for brief or longer periods in the environment. Salmonellosis is a zoonosis, whereas *E. coli* and *Klebsiella* spp. are common non-pathogenic inhabitants of the gastrointestinal tract that may become opportunistic pathogens when the immune system is impaired or normal defence barriers are breached (e.g. surgery, trauma, underlying disease).

2.5 Antimicrobial usage and resistance in human health care

2.5.1 Importance of the drugs for human medicine

Impact on disease burden

Since their introduction, now more than 60 years ago, antimicrobial drugs have substantially reduced the disease burden caused by infectious diseases that were previously widespread, untreatable and often fatal. Together with improved hygiene, housing, nutrition and vaccination programmes, they have contributed to a longer and healthier life of millions of people (WHO, 2002).

Classification

Drugs are classified according to the the Anatomical Therapeutic Chemical (ATC) classification system of the WHO (www.whocc.no). Within this classification, the majority of antibacterial drugs belongs to class J01, the antibacterials for systemic use. The ATC system also includes the Defined Daily Dose (DDD), which is a measurement of drug consumption based on the usual daily dose of each antibiotic in adults

(see also section 2.5.2). There are several classes of antibacterial drugs available for human and veterinary medicine, see also Appendix. This classification is based on similarities in their molecular structure. Some classes are solely used in either human or veterinary medicine, but the majority is used in both.

Critically important antimicrobial drugs

During expert meetings organized by the WHO, a list of antimicrobial drugs that are deemed critically important in the treatment of human disease was developed (WHO, 2007). This list was developed as a risk management strategy to determine which antimicrobial drugs should be banned or should be used only limited in the non-human sector to contain the problem of antimicrobial resistance. The following (sub)classes of antimicrobials were classified as critically important for human medicine:

- beta-lactams: penicillins, cephalosporins (third and fourth generation) and (carba)penems
- aminoglycosides
- tetracyclines (tigecycline only)
- macrolides and ketolides
- glycopeptides
- quinolones
- lipopeptides
- oxazolidinones
- streptogramins
- ansamycins
- drugs used solely to treat tuberculosis or other mycobacterial diseases

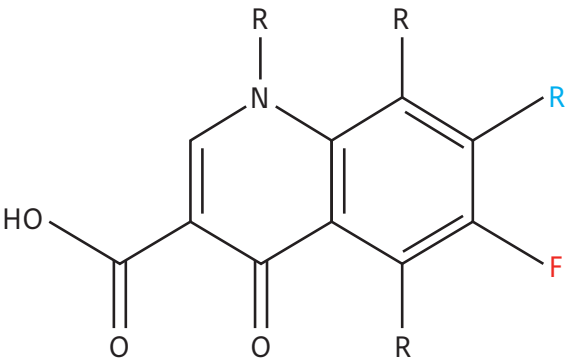
A comparable list of critically important antimicrobials for food animal therapy was developed by the OIE (OIE, 2007).

Quinolones

Quinolones are a broad and expanding class of antimicrobials that contain a dual-ring structure with nitrogen, a carbonyl and a carboxyl group in the first ring (Figure 3). Quinolones with fluorine in the second ring are named fluoroquinolones. Quinolones can be categorized in four broad groups: the first group being the older quinolones with activity against Gram-negative enterobacteria and group 2, 3 and 4 the fluoroquinolones with ever increasing spectrum and an improved activity against Gram-positive cocci and anaerobes (Finch, 2003).

Quinolones are bactericidal antimicrobial drugs and their mechanism of action is the inhibition of bacterial DNA replication and transcription.
The first quinolones were derived from attempts to synthesize the anti-malarial drug chloroquine. Nalidixic acid was the first quinolone introduced for clinical use in 1962, several others quickly followed. Some quinolones have been withdrawn from clinical use due to toxicity problems. Their therapeutic use comprises a wide range of

Figure 3 Essential structure of all quinolone antibiotics: the blue drawn remainder of R is usually piperazine; if the connection contains fluorine (red), it is a fluoroquinolone (Wikipedia, 2009)



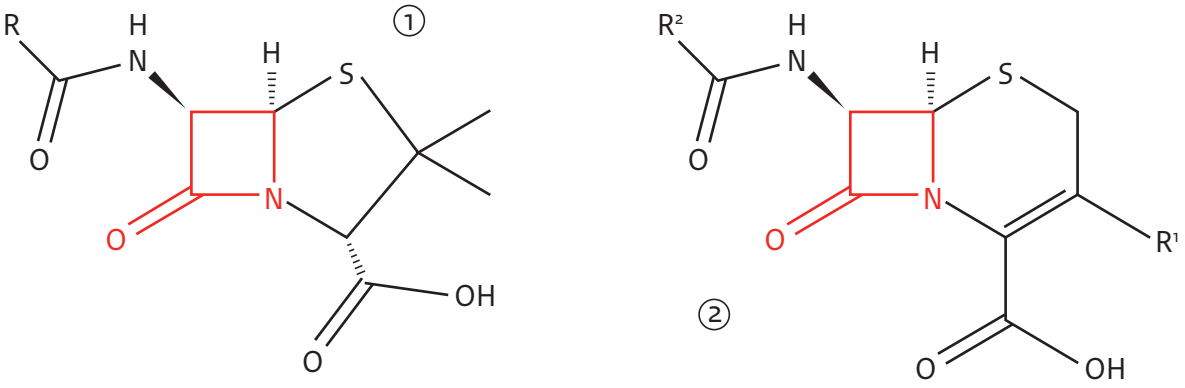
disorders, e.g. genitourinary tract infections, respiratory disorders, sinusitis, ocular infections, etcetera. (Finch, 2003). Quinolones are classified as critically important for both human and animal health and should be addressed as the highest priority for the development of risk management strategies with respect to antimicrobial resistance (FAO/WHO/OIE, 2008).

Beta-lactams

Beta-lactams form a broad class of antimicrobials that contain a beta-lactam ring in their molecular structure (Figure 2). This class includes the subclasses penicillins, cephalosporins, monobactams, and carbapenems.

Beta-lactam antibiotics are bactericidal antimicrobial drugs and their mechanism of action is the inhibition of the bacterial cell wall synthesis. Penicillin, discovered by Alexander Fleming in 1928, was the first antimicrobial drug to be introduced for large scale treatment. Production of this antimicrobial drug started during World War II for treatment of infected war wounds. Soon, derivatives of penicillin were developed to treat a wider range of infections. Beta-lactam antibiotics are the most frequently used antimicrobial drugs in hospitals and primary health care (SWAP. NethMap, 2009). They are used to treat a wide range of infections, e.g. urinary tract infection, wound infection, sepsis, respiratory infections, meningitis, etcetera. They are known to be among the safest antibiotics to be used therapeutically, but unfortunately allergic reactions do occur frequently (Finch, 2003). The subclasses penicillins, cephalosporins (third and fourth generation) and (carba)penems are categorized as critically important for human health. The penicillins and third and fourth generation cephalosporins are also classified as critically important for food animal medicine (FAO/WHO/OIE, 2008). Moreover, experts defined that the cephalosporins should be addressed as the highest priority for the development of risk management strategies with respect to antimicrobial resistance (FAO/WHO/OIE, 2008).

Figure 4 Core structure of penicillins (1) and cephalosporins (2). Beta-lactam ring in red (Wikipedia, 2009).



2.5.2 Usage

Collection of consumption data

In the Netherlands, all antimicrobial drugs are prescription-only medicines delivered to patients by either community pharmacies, hospital pharmacies or general practitioners with their own pharmacy, who serve approximately 8.4% of the Dutch population (Van Batenburg-Eddes et al., 2002). Self-medication does occur, but is more prominent in southern and eastern European countries (Grigoryan et al., 2008). Information on the human consumption of antimicrobial drugs is collected by the Dutch Foundation on Antibiotic Policy (Stichting Werkgroep Antibiotica Beleid, SWAB). The data are presented annually in the yearly NethMap reports which can be downloaded from www.swab.nl. Data on the consumption of antimicrobial drugs in hospitals are obtained from hospital pharmacies. Antimicrobial drug use in the community (primary health care) is obtained from approximately 90% of all community pharmacies and is provided by the Foundation for Pharmaceutical Statistics (Stichting Farmaceutische Kengetallen, SFK), (Prins et al., 2008).

Measures: DDD and DID

Antimicrobial drug usage is expressed in the standardized measure Defined Daily Dose (DDD). DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults (WHO, 2009) and is used to compare drug consumption between different countries. To compare usage over time, the usage in primary health care is expressed as DDD per 1000 inhabitants per day (DID) and in hospitals as DDD per 100 patient-days and in DDD per 100 admissions (Filius et al., 2005).

Consumption of antimicrobial drugs (Sources: SFK and SWAB)

The overall use of antimicrobial drugs for systemic use in primary health care in 2008 was 11 DID. In hospitals, the overall usage of systemic antimicrobial drugs in 2007 was 61 DDD/100 patient-days and 335 DDD/100 admissions. In primary health care, the main classes used are tetracyclines (17%, mainly doxycycline) and beta-lactams (39%) of which penicillins with extended spectrum (25% of total use, mainly amoxicillin) and combinations of penicillins with beta-lactamase inhibitors (15%, mainly co-amoxiclav) are the main subclasses (Figure 5). In hospitals, the main class is the beta-lactams (62%) of which combinations of penicillins with beta-lactamase inhibitors (24%, mainly co-amoxiclav) and cephalosporins (14%) are the main subclasses (Figure 6).

Trends in antimicrobial drug usage (SWAB. NethMap, 2009)

From 1999-2008, the overall use of antimicrobial drugs for systemic use in primary health care gradually increased from 10 to 11 DID (Table 2).

From 2003-2007, the overall use of antibiotics for systemic use in hospitals increased from 52 to 61 DDD/100 patient-days (Table 3). However, the mean antibiotic use per hospital patient remained constant (333 and 335 DDD/100 admissions in 2003 and 2007 respectively). The following trends in antibiotic use per (sub)class could be seen:

- Mean use per patient increased for cephalosporins, carbapenems, lincosamides, glycopeptides and nitrofurantoin.
- Mean use per patient remained the same, but due to more admissions the use per hospital increased for penicillins with extended spectrum, combinations of penicillins, macrolides and fluoroquinolones.
- Mean use per patient decreased, but due to an increase in admissions the use per hospital remained constant for tetracyclines, beta-lactamase resistant penicillins, combinations of sulphonamides and trimethoprim and group 1 quinolones.
- Mean use per patient decreased for trimethoprim and derivatives and aminoglycosides.

Figure 5 Distribution of the use of antibiotics for systemic use in primary health care, 2008 Source: SFK, figure obtained from (SWAB. NethMap, 2009)

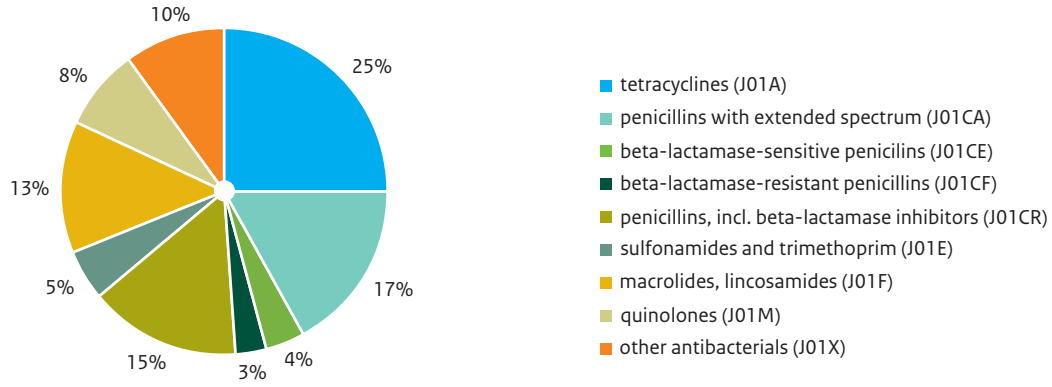


Figure 6 Distribution of the use of antibiotics for systemic use in hospitals, 2007. Source: SWAB, figure obtained from (SWAB. NethMap, 2009)

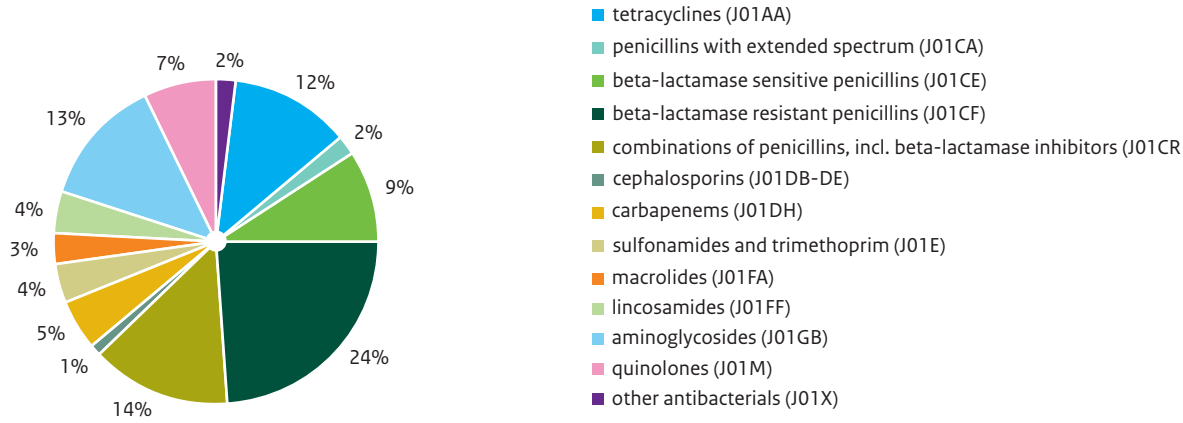


Table 2 Data on the use of antibiotics for systemic use (J01) in primary care (DDD/1000 inhabitants-days), 1999 and 2008. Source: SFK, table adapted from (SWAB. NethMap, 2009)

ATC Group*	Therapeutic group	Year	
		1999	**2008**
J01AA	Tetracyclines	2.49	2.67
J01CA	Penicillins with extended spectrum	2.05	1.92
J01CE	Beta-lactamase sensitive penicillins	0.52	0.42
J01CF	Beta-lactamase resistant penicillins	0.23	0.36
J01CR	Penicillins + beta-lactamase-inhibitors	1.04	1.72
J01D	Cephalosporins	0.10	0.04
J01EA	Trimethoprim and derivatives	0.30	0.22
J01EC	Intermediate-acting sulphonamides	0.00	0.00
J01EE	Sulphonamides + trimethoprim	0.46	0.36
J01FA	Macrolides	1.17	1.37
J01FF	Lincosamides	0.04	0.11
J01GB	Aminoglycosides	0.00	0.03
J01MA	Fluoroquinolones	0.85	0.90
J01MB	Other quinolones	0.04	0.02
J01XB	Polymyxins	0.02	0.00
J01XE	Nitrofuran derivatives	0.64	1.14
J01XX05	Methenamine	0.06	0.02
J01	Antibiotics for systemic use (total)	10.02	11.33

* From the 2008 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 3 Data on the use of antibiotics for systemic use (J01) in hospitals (DDD/100 patients-days and DDD/100 admissions), 2003 and 2007. Source: SWAB, table adapted from NethMap (SWAB. NethMap, 2009)

ATC Group*	Therapeutic group	Year			
		DDD/100 patient-days		DDD/100 admissions	
		2003	2007	2003	2007
J01AA	Tetracyclines	1.4	1.4	8.8	7.7
J01CA	Penicillins with extended spectrum	6.0	7.3	38.6	40.3
J01CE	Beta-lactamase sensitive penicillins	1.2	1.2	7.8	6.8
J01CF	Beta-lactamase resistant penicillins	5.4	5.6	34.6	31.0
J01CR	Penicillins + beta-lactamase-inhibitors	12.1	14.0	77.7	77.3
J01DB-DE	Cephalosporins	6.5	8.4	42.0	46.3
J01DF	Monobactams	0.0	0.0	0.0	0.0
J01DH	Carbapenems	0.5	0.8	3.3	4.4
J01EA	Trimethoprim and derivatives	0.5	0.5	3.1	2.9
J01EC	Intermediate-acting sulphonamides	0.1	0.1	0.8	0.4
J01EE	Sulphonamides + trimethoprim	2.3	2.3	14.4	12.7
J01FA	Macrolides	2.4	2.7	15.4	14.8
J01FF	Lincosamides	1.6	2.1	10.2	11.5
J01GB	Aminoglycosides	2.5	2.5	15.8	14.0
J01MA	Fluoroquinolones	6.4	7.6	41.0	41.9
J01MB	Other quinolones	0.1	0.0	0.6	0.2
J01XA	Fluoroquinolones	0.5	1.0	3.4	5.3
J01XB	Polymyxins	0.1	0.1	0.5	0.7
J01XC	Steroid antibacterials (fusidic acid)	0.0	0.0	0.2	0.1
J01XD	Imidazole derivatives	1.6	1.8	10.1	9.9
J01XE	Nitrofuran derivatives	0.7	1.1	4.7	6.2
J01XX05	Methenamine	0.0	0.0	0.2	0.1
J01XX08	Linezolid	0.0	0.0	0.1	0.2
J01	Antibiotics for systemic use (total)	51.9	60.9	333.2	335.0

* From the 2008 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Comparison with usage in other European countries

Information on antimicrobial drug consumption in European memberstates, candidate countries and free-trade countries is collected by the European Surveillance of Antimicrobial Consumption project (ESAC). Usage is expressed in DID. In 2006, the total outpatient use ranged from 27.91 DID in France to 9.58 DID in Russia (Figure 7). The Netherlands had the second lowest score, 10.85 DID (Coenen et al., 2009). In general, antimicrobial drug usage seems to be higher in southern European countries (Goossens, 2009).

Quinolones

In the Netherlands, the total use of quinolones in primary health care increased slightly, from 0.89 in 1999 to 0.91 DID in 2008. Ciprofloxacin was the most commonly used fluoroquinolone of which the use more than doubled (Figure 8). In hospitals, the total use of quinolones increased from 6.5 in 2003 to 7.6 DID and from 41.6 to 42.1 DDD/100 admissions in 2007. The use of ciprofloxacin increased, while the use of the other quinolones decreased, or remained relatively low (Figure 9). Within Europe, quinolone use ranged from 3.46 DID in Italy to 0.37 DID in Denmark. The use in the Netherlands was fairly low with 0.91 (the fifth lowest use, Figure 7), (Coenen et al., 2009).

Beta-lactams

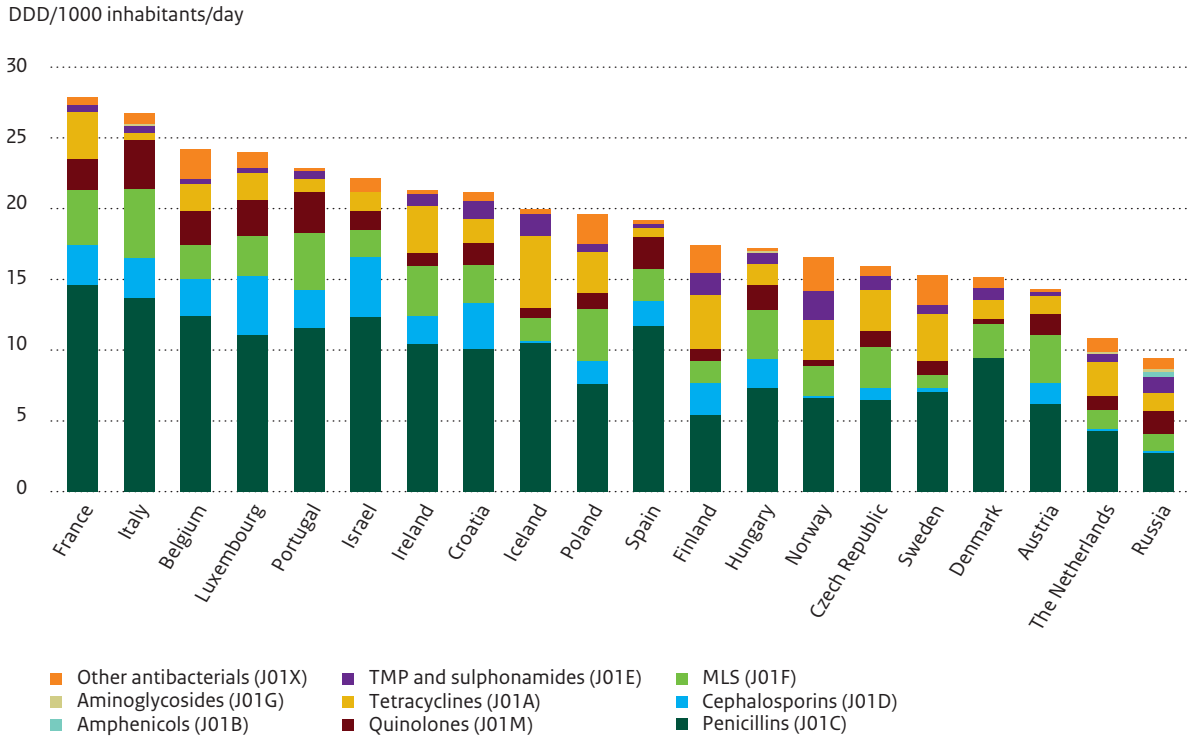
The total use of beta-lactams in primary health care increased from 3.94 in 1999 to 4.44 DID in 2008. The use of amoxicillin slightly decreased, while the use of co-amoxiclav almost doubled (Figure 10). In hospitals, the total use of beta-lactams increased from 6.5 in 2003 to 7.6 DDD/100 patient-days and from 41.6 to 42.1 DDD/100 admissions in 2007. The use of co-amoxiclav increased from 1999-2006, but decreased in 2007 (Figure 11). The use of most other penicillins also decreased in 2007. The use of first and third generation cephalosporins increased, while second generation cephalosporins stabilized (Figure 12). Within Europe, penicillins and cephalosporins use ranged from 14.61 DID in France to 2.68 DID in Russia and 4.26 DID in Israel and 0.03 DID in Denmark, respectively. In the Netherlands, the use of penicillins was 4.32 DID and of cephalosporins 0.05 DID (both second lowest use in Europe according to ESAC, Figure 7).

2.5.3 Resistance

The threat of resistance

The emergence and spread of bacteria resistant to cheap and effective first-choice antimicrobial drugs represents a

Figure 7 Outpatient antibiotic use in DID in 20 European countries in 2006. ESAC data as presented in Coenen et al. (Coenen et al., 2009)



major threat to the health of the human population. Resistance makes first-choice drugs ineffective. Consequently, treatment has to be switched to other drugs which are often more expensive and sometimes also more toxic. In the near future, for some diseases there will no longer be any effective drugs available (WHO, 2002).

Collection of susceptibility data

In the Netherlands, information on antimicrobial drug resistance is collected by the SWAB and presented annually in NethMap. Susceptibility data in the community are collected on a selection of medically important bacteria. In addition, susceptibility data from unselected hospital department and outpatient clinics are collected through the national Infectious Diseases Information System for Antibiotic Resistance (ISIS-AR) and data from specific hospital wards (intensive care units (ICUs), urology services and pulmonology services) are obtained (SWAB. NethMap, 2009).

Figure 8 Use of quinolones for systemic use in primary health care, 1997-2008. Source: SFK, figure obtained from NethMap (SWAB. NethMap, 2009)

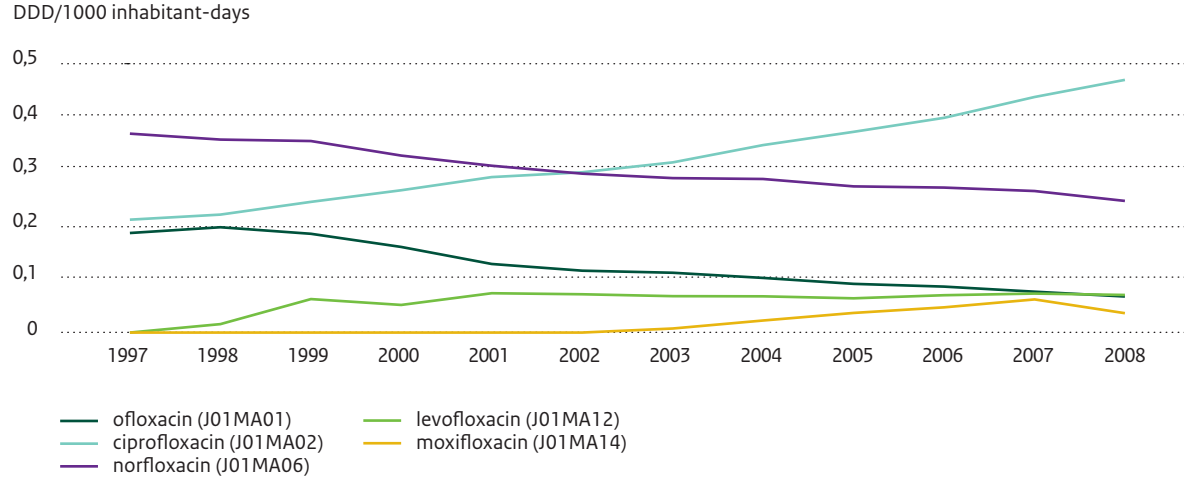


Figure 9 Use of fluoroquinolones in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B). Source: SFK, figure obtained from NethMap (SWAB. NethMap, 2009)

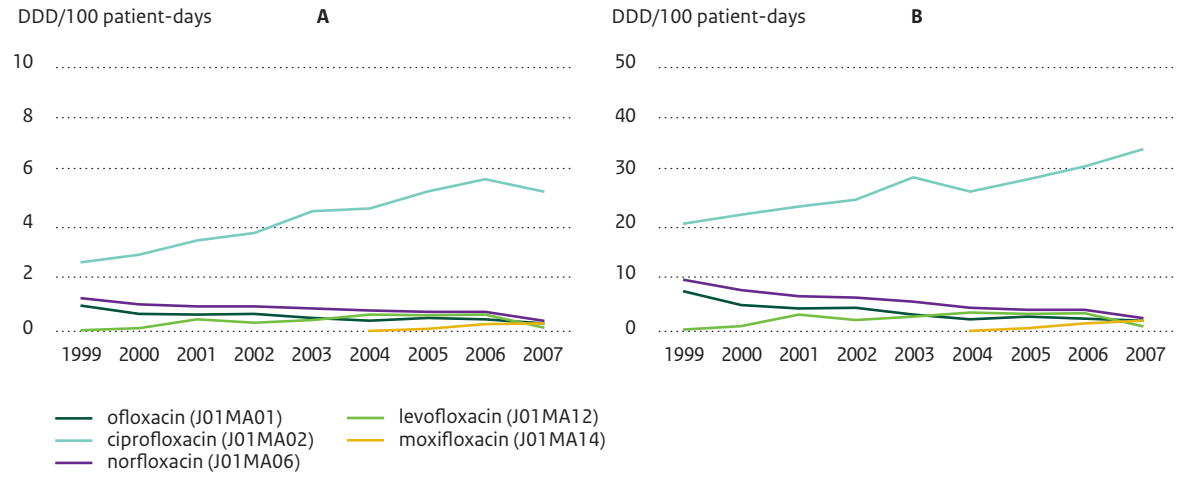


Figure 10 Use of amoxicillin and co-amoxiclav in primary health care, 1997-2008. Source: SFK, figure obtained from NethMap (SWAB. NethMap, 2009)

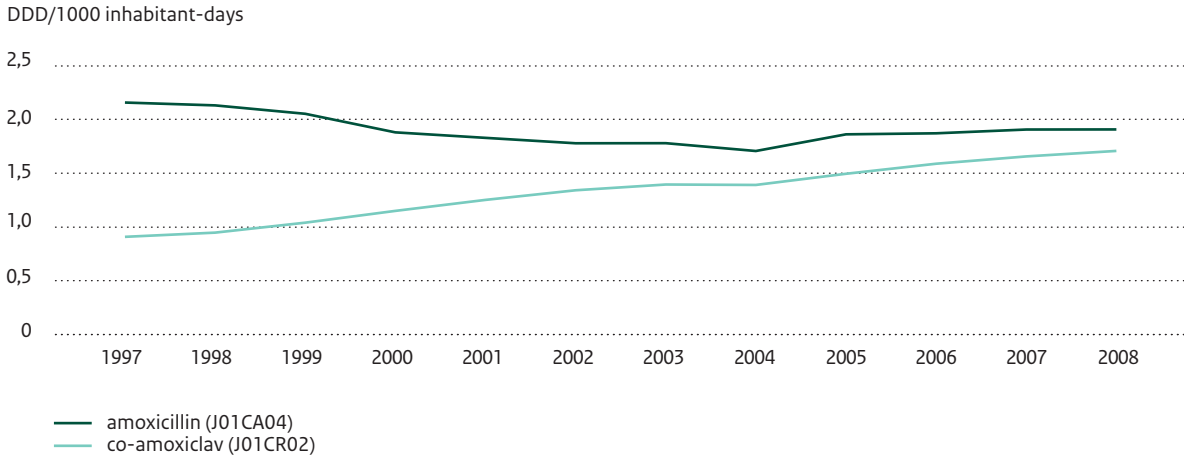


Figure 11 Use of penicillins in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B). Figure obtained from NethMap (SWAB. NethMap, 2009)

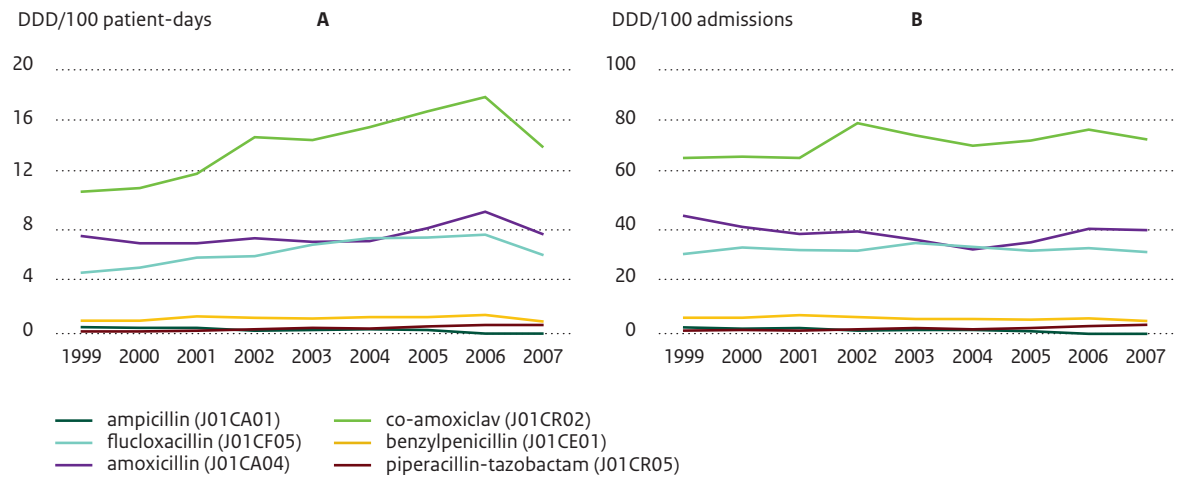
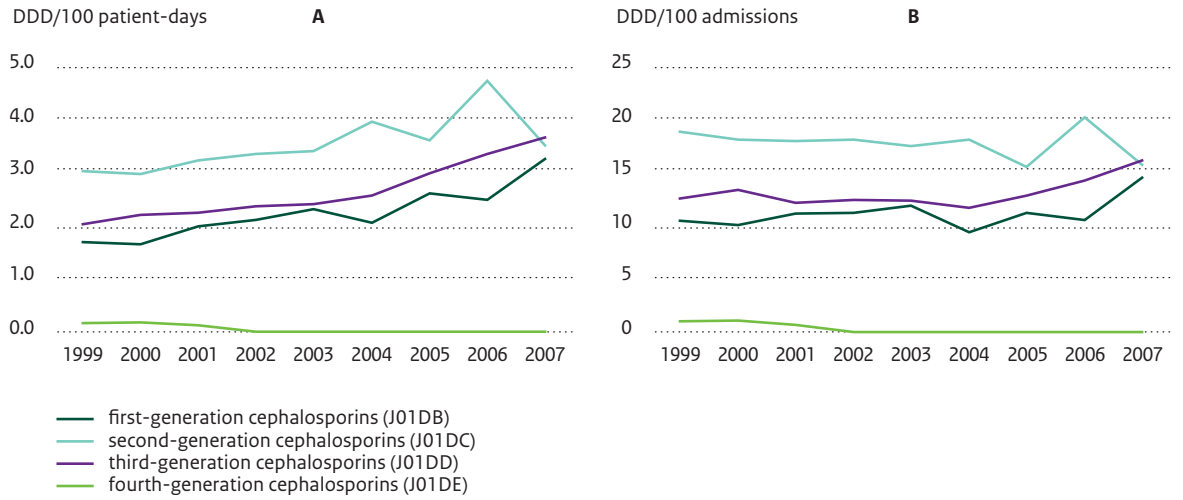


Figure 12 Use of cephalosporins in hospitals, expressed as DDD/100 patient-days (A) and DDD/100 admissions (B). Figure obtained from NethMap (SWAB. NethMap, 2009)



Trends in antimicrobial drug resistance (SWAB. NethMap, 2009)

In general, the majority of antimicrobial resistance levels in and outside hospitals is rising or is stable depending on the drug-bug combination. In clinical isolates of *Escherichia coli* obtained from unselected hospital departments, ICUs and urology services, increasing resistance levels to commonly used antimicrobials such as amoxicillin and ciprofloxacin were found. Resistance to penicillins, cephalosporins and gentamicin was highest in ICUs and multiresistance (resistance to three or more antibiotics classes) was found to be increasing in these strains. Multiresistance is also common among *Staphylococcus aureus* strains from ICUs. Resistance levels in *Neisseria gonorrhoeae* are alarmingly high and are still rising, whereas resistance levels of *Mycobacterium tuberculosis* are stable at a low level.

Comparison with other European countries (EARRS, 2009)

Data on antimicrobial drug resistance of major invasive bacteria in Europe are collected by the European Antimicrobial Resistance Surveillance System (EARSS, www.rivm.nl/earss). This is an international network of national surveillance systems in 33 European countries. The rates of antimicrobial resistance show wide variations among European countries. In general, higher resistance levels are found in southern European countries. For most bacteria under surveillance, resistance levels in the Netherlands are relatively low, in particular for *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*.

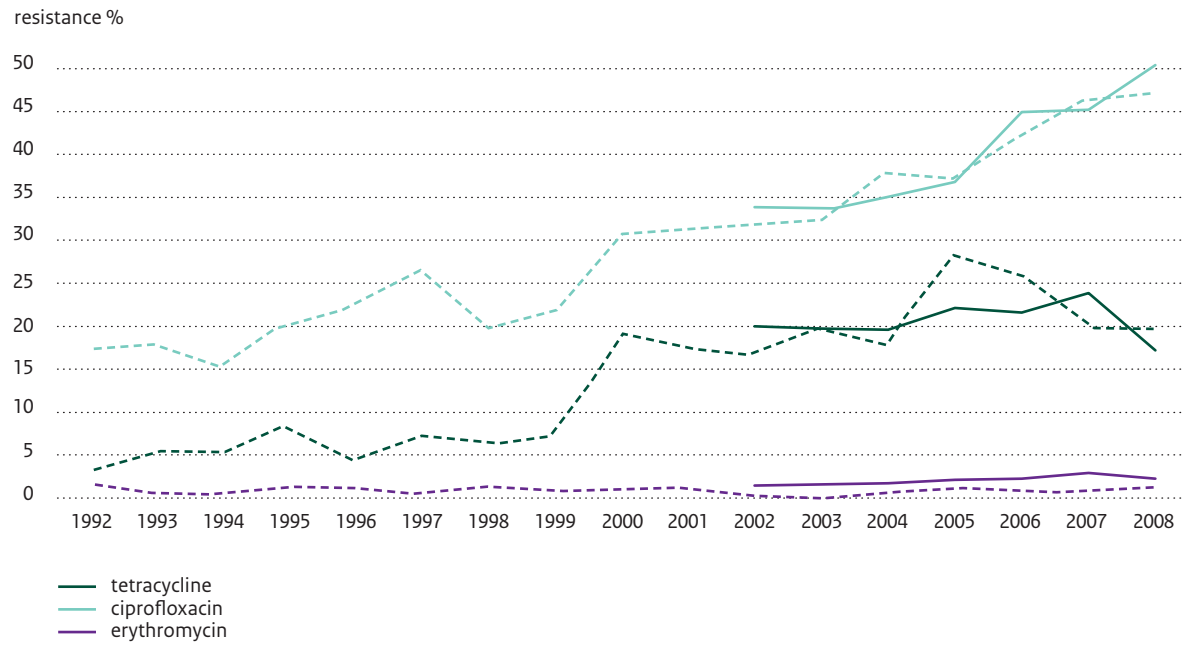
Quinolone resistance in *Campylobacter jejuni*

Treatment with quinolones quickly selects for very high levels of resistance in *Campylobacter jejuni* most likely due to mutations in the DNA gyrase and DNA topoisomerase IV genes (Alfredson and Korolik, 2007; McDermott et al., 2002; Van Boven et al., 2003). In the Netherlands, resistance of domestically acquired *C. jejuni* to ciprofloxacin, a fluoroquinolone, has increased from on average 32.7% in 2002-2005 to 45.4% in 2006-2008. Ciprofloxacin resistance in travel related cases was higher: on average 53.5% in 2002-2005 to 62.9% in 2006-2008 (MARAN-2008). The resistance to tetracycline is also high and rising; erythromycin resistance remains rare (Figure 13). In European member states, the overall resistance to ciprofloxacin in *C. jejuni* is high and rising from 37% in 2005 to 44.2% in 2006. Multidrug resistance in *C. jejuni* (resistance to at least 4 antimicrobials) was 8.4% in 2006 (EFSA, 2007). Antibiotic treatment of patients with acute infectious diarrhoea is restricted to individuals with severe illness, dysentery and/or a predisposition to complications. As a consequence of the high quinolone resistance levels in *Campylobacter*, the guidelines for empirical treatment of acute infectious diarrhoea were adapted; it is advised to use oral azithromycin or, in case of intravenous treatment, a combination of ciprofloxacin and erythromycin (Bos et al., 2006).

LA-MRSA

Livestock-associated MRSA (LA-MRSA) has acquired the *mecA* gene, which codes for a variant of the penicillin binding protein (PBP) that has a low affinity for beta-lactam antibiotics. This renders this bacterium resistant to all beta-lactam antibiotics. LA-MRSA is a multidrug resistant bacterium; besides resistance to beta-lactams, human isolates were also found to be resistant to tetracycline, erythromycin, ciprofloxacin, lincomycin, gentamicin, kanamycin, doxycycline, tobramycin and clindamycin (Van Duijkeren et al., 2008; Van Loo et al., 2007; Wagenaar and Van de Giessen, 2009). Vancomycin

Figure 13 Trends in resistance (%) of Campylobacter spp. isolated from humans (1992-2008) at the regional Public Health Laboratories (400-700 isolates per year), obtained from: MARAN-2008



resistance has not been found yet. It is advised that therapy of severe infections should be started either with an intravenous glycopeptide or with oral ciprofloxacin, possibly combined with rifampicin or linezolid (Renders et al., 2007). Moreover, in our country LA-MRSA is often still susceptible to co-trimoxazole. Information on trends in resistance and comparisons with other European countries is not available.

ESBL-positive *Escherichia coli* and *Klebsiella* spp.

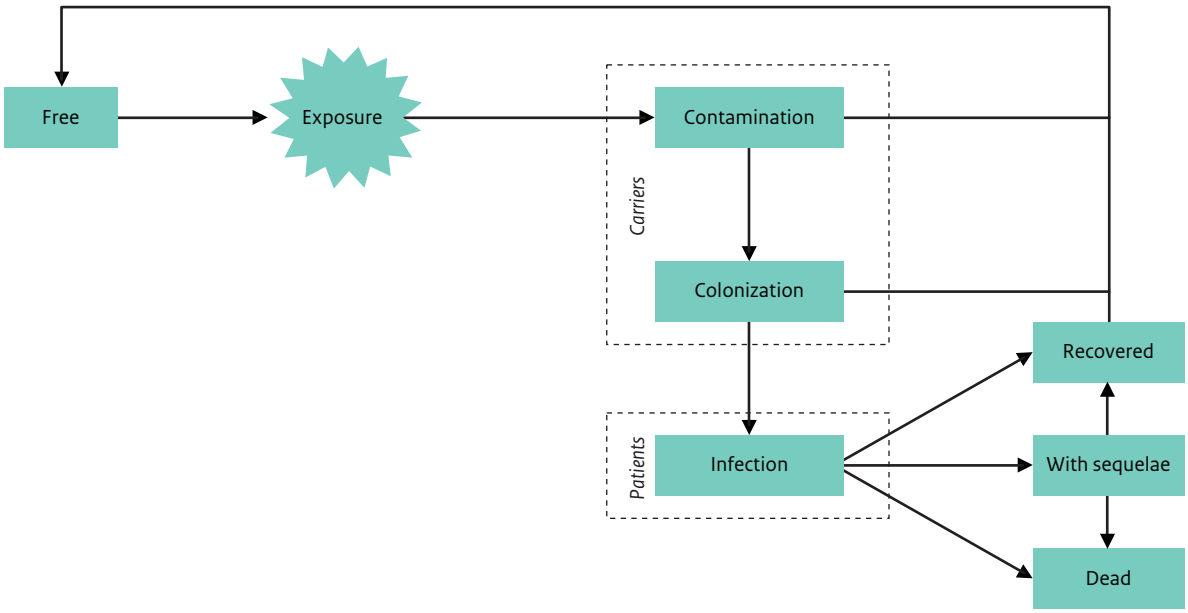
Extended-spectrum beta-lactamases (ESBLs) are enzymes produced by Gram-negative bacteria that are capable of inactivating penicillins, cephalosporins and aztreonam. In surveillance studies, the production of ESBLs is often not tested, but resistance to third generation cephalosporins (e.g. cefotaxime and ceftazidime) is assessed, which is a good indicator of ESBL production. In the Netherlands, ESBL production was detected in *E. coli* and *Klebsiella pneumoniae* isolates from patients at the ICU of 8 hospitals in the period 1998-2005. ESBL production was found in 0.7-0.9% of the *E. coli* and in 5.5-6.7% of the *Klebsiella pneumoniae* isolates. The number of ESBL-positive isolates in this study increased from 2002 onwards (Oudhuis et al. in NethMap, 2008). The prevalence of third generation cephalosporin-resistant *E. coli* blood isolates increased from 0.5% in 2001 to 4.6% in 2008, the prevalence of third generation cephalosporin-resistant *K. pneumoniae* isolates increased from 3.9% in 2005 to 7.6% in 2008 (EARSS-database). In comparison to other European countries, the resistance to third generation cephalosporins in *K. pneumoniae* and *E. coli* is still low, but it is rising significantly. Carbapenems are still effective to treat infections with *K. pneumoniae* in most countries, but the emergence of carbapenemase producing strains is threatening this therapeutic option (EARRS, 2009).

2.6 Adverse health consequences in humans

The degree of adverse health consequences caused by bacteria is dependent on the virulence characteristics of the bacterium, the transmission route, the dose of bacteria and the disease susceptibility of the host. Antimicrobial resistance can be viewed as a virulence trait of bacteria that counteracts antimicrobial treatment of infections that causes a delay in appropriate antimicrobial treatment, decreased antimicrobial effectiveness, increased antimicrobial toxicity, improper antimicrobial dosing and/or increased need for surgery and other invasive procedures (Maragakis et al., 2008). As a consequence, a longer duration of illness, increased frequency of bloodstream and internal infections, prolonged hospital stay, or increased mortality may occur. Moreover, use of antimicrobials that disturb the microbial commensal flora may result in infections with antimicrobial-resistant bacteria that would otherwise not have occurred (FAO/OIE/WHO, 2003).

In this section, available information on risk populations and the type and severity of adverse health consequences caused by the example agents will be summarized. Focus will be on both infectious disease and bacterial carriage.

Figure 14 Flow diagram of possible outcomes of exposure to bacteria



Carriers of antimicrobial-resistant bacteria often have a higher probability of becoming diseased (Wertheim et al., 2005) and may play a role in the spread of antimicrobial resistance. They will therefore be discussed as a separate risk group for each bacterium if relevant. The set up of this section will be as follows. A brief summary of the mechanisms leading to carriage or infectious disease in humans is given in section 2.6.1. Some populations have a higher risk of carriage or becoming diseased than others. Information on the risk populations for each of the four example bacteria is summarized in section 2.6.2. Finally in section 2.6.3, the type and severity of adverse health effects caused by the four example bacteria is described.

2.6.1 Mechanisms leading to infectious disease

Hereafter the mechanisms leading to infectious disease in humans are briefly described. In Figure 14, the possible outcomes of exposure to bacteria are presented schematically.

Exposure

People can be exposed to bacteria in various ways. Exposure sources can either be people or animals that shed the bacterium (Baran Jr et al., 2002; Wulf et al., 2008a) or environmental or food-related sources (Aarestrup et al., 2008) that contain the bacterium.

Contamination

If the bacterium has successfully been transmitted from the exposure source to the body, usually to the mucosa of the respiratory or gastrointestinal tract or to the skin, the host has become contaminated. Depending on the suitability of this new environment, the bacterium will be able to colonize or not. In the latter case, the host will be a transient carrier. Transient carriers will not experience any adverse health consequences of the presence of the bacterium. If not permanently exposed to the infectious source, they will loose the bacterium and become free again.

Colonization

If the host-bacterium interaction characteristics are favourable, the bacterium will adhere to the host tissue and start to multiply, which is referred to as colonization. Depending on the number of bacteria (dose), the characteristics and virulence of the bacterium, the location of colonization, and the immune response of the host, colonization may either lead to an acute infection or result in (semi-)permanent carriage without adverse health consequences.

Infection

In case of an infection the bacteria are able to invade and damage host tissue. An infection evokes an immune response and may cause noticeable disease symptoms (symptomatic infection i.e. disease). Carriers may become infected when circumstances change, e.g. when normal defensive barriers are breached (intravascular lines, urinary catheters) or the immune system is compromised due to underlying illness. The intensity of the adverse health effects determines the need of medical intervention, e.g. the use of antimicrobials.

Transfer of resistance genes

Besides direct contamination, colonization and infection

with antimicrobial-resistant bacteria, antimicrobial resistance may also be transferred to a new host by conjugation, transformation or transduction of resistance genes from bacteria acquired from an exposure source to bacteria present in the commensal flora of the host (see also section 2.1). It is not sufficiently clear which host factors play a role in these processes (Hunter et al., 2008) and how often this will result in infectious disease.

2.6.2 Populations at risk

Carriers versus patients

As stated above, carriers do not directly experience any adverse health consequences of carriage, but may contribute to transmission of antimicrobial-resistant bacteria and hence may contribute to economic consequences (e.g. screening costs). *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus aureus* usually are non-virulent bacteria that may colonize permanently. and *E. coli* belong to the normal inhabitants of the commensal flora of the gastrointestinal tract, whereas for *S. aureus* it is estimated that 20-55% of the human population carries this bacterium in the nasal flora (Kluytmans et al., 1997). *Klebsiella* spp. can be found in the nasopharynx and in the intestinal tract (Podschun and Ullmann, 1998). These bacteria colonize without evoking an effective immune response or causing any adverse health consequences unless normal defense barriers are breached. In contrast, *Campylobacter jejuni* usually causes acute infection. Carriage and infections will be discussed separately for each bacterium.

Health care institutions versus community

In this report, populations at risk in health care institutions and in the community are discussed separately for each bacterium. Health care institutions are defined as intramural care for patients, elderly, physically or mentally disabled persons, i.e. hospitals and long-term care facilities. Populations in health care institutions and communities differ in antimicrobial use and disease susceptibility (due to age, underlying disease, facilitated entrance of bacteria by (surgical) wounds, ventilators, catheters, etcetera), and the populations may be exposed to different sources of infection. Moreover, some bacterial strains have successfully adapted to the hospital environment where they have become endemic and hence are difficult to control. Obviously, bacteria and resistance genes may be transferred from health care institutions to the community and vice versa and hence the distinction between typical health care and community associated infections has become less strict.

Quinolone-resistant *Campylobacter jejuni*

Campylobacter jejuni infections are mainly associated with exposure in the community (EFSA, 2009d).

Risk groups for carriage

The mean excretion period of *C. jejuni* after an episode of illness is 3-5 weeks; long-term carriage has been observed only in patients with hypogammaglobulinemia and AIDS (Skirrow and Blaser, 2000). Assymptomatic infections are common; it is assumed that the bacterium-specific immune response limits disease and leads to the development of protective immunity (Havelaar et al., 2009).

Risk groups for infection in the community

Campylobacter infections occur in all age groups, specific risk groups identified are young children (0-5 years) and young adults (20-35 years) (Havelaar, 2001), pregnant women (Smith, 2002) and professionals working with raw meat or poultry (Adak et al., 1995; Price et al., 2007b; Studahl and Andersson, 2000). Several risk factors for campylobacteriosis have been identified (Carrique-Mas et al., 2005): the consumption of undercooked chicken, undercooked beef, raw milk or contaminated water, eating at a restaurant, eating at barbecues, poor kitchen hygiene, traveling abroad and contact with pets (Gallay et al., 2008; Kapperud et al., 2003; Studahl and Andersson, 2000). In the Netherlands, 20-40% of the laboratory cases were estimated to be attributable to the consumption of undercooked chicken (Havelaar et al., 2005). However, approximately 50% of the *Campylobacter* cases cannot be attributed to any of the known risk factors (Janssen et al., 2008). Recent international studies using microbial subtyping have shown that approximately 50-80% of all human cases of *Campylobacter jejuni* can be attributed to the chicken reservoir (EFSA, 2009d); no data on subtyping of fluoroquinolone-resistant strains have yet been published.

Risk groups for infection in health care institutions

Campylobacter infections are mainly associated with exposure in the community. Patient groups that are more at risk of *Campylobacter* infections are those with hypo- or agammaglobulinemia or AIDS (Freeman and Holland, 2007; Sorvillo et al., 1991).

LA-MRSA

Whereas LA-MRSA carriage is mainly associated with exposure in the community, severe infections mainly occur in healthcare settings.

Risk groups for carriage

LA-MRSA occurs significantly more often in people in direct contact with livestock; this risk increases with intensity of the contact with the animals (Wagenaar and Van de Giessen, 2009). Risk groups that have already been identified include pig and veal calf farmers and their families, as well as veterinarians and slaughterhouse employees (Khanna et al., 2008; Meemken et al., 2008; Moodley et al., 2008; Mulders et al., 2010; Van Cleef et al., 2010b; Van den Broek et al., 2009; Van Loo et al., 2007;

Wulf et al., 2006; Wulf et al., 2008a). Prevalences reported from the Netherlands varied from 23% in pig farmers in an initial pilot (Voss et al., 2005) to 29% in pig farmers and pig farm workers and 14% in pig farm residents (including farmers and co-workers) in a more extensive study (Van den Broek et al., 2009). The prevalence found in Dutch veterinarians and veterinary students was 4.6% (Wulf et al., 2006). In veal calf farming, prevalences reported were 32% in farmers and 8% in their family members (Graveland et al., 2008). One case report of LA-MRSA in people related to broiler farms (Leenders, 2007) and findings of LA-MRSA in healthy broilers in Belgium (Nemati et al., 2009; Persoons et al., 2009) suggest that also broiler farmers may be at risk of carriage. In broiler and pig slaughterhouses, 13.8% and 15.1% of the employees in direct contact with live animals were carriers of LA-MRSA (Mulders et al., 2010; Van Cleef et al., 2010b). Also other occupational groups in direct contact with live animals may be at risk of carriage with LA-MRSA, e.g. drivers of animal transports and poultry catchers. In contrast, people occupied in the slaughterhouse that had no contact with live animals and people that professionally have a high exposure to meat products, but not to live animals, did not have a higher prevalence of LA-MRSA carriage than the general Dutch population (Wagenaar and Van de Giessen, 2009). Health care workers (HCW) in contact with pigs and cattle had a tenfold higher MRSA carriage than the HCW control group, but this difference was not significant (Wulf et al., 2008b). LA-MRSA has not spread to people living in municipalities with a high density of pig farms (Van Cleef et al., 2010a).

Risk groups for infection in the community

Reported LA-MRSA cases in the community were related to occupational groups in direct contact with livestock (Declercq et al., 2008; Huijsdens et al., 2006).

Risk groups for infection in health care institutions

Carriers of LA-MRSA admitted to a hospital are at risk of acquiring an infection with this bacterium. They may also cause the spread of this bacterium within the hospital, although it was found that secondary spread was only limited compared to HA-MRSA (Bootsma et al., 2010; Wassenberg et al., 2010) and concerned only HCW and not patients (Wassenberg et al., 2008). Since July 2006, all people in close contact with live pigs or veal calves are screened for MRSA at admission to the hospital (www.wip.nl). In 2007 and 2008, 92 of the MRSA isolates sent to the Dutch Staphylococcus reference centre at RIVM were most likely obtained from patients with an actual infection (Haenen et al., 2009). In 2007, one isolate was sent in by a nursing home, 41 were sent in by hospitals, all others were unknown. In 2008, one isolate was sent in by a nursing home, 52 were sent in by hospitals, 7 from the patients home and of 32 isolates the origin was unknown (Haenen, personal communication). Risk factor analysis on patients with LA-MRSA has not been performed yet. Reported clinical cases of LA-MRSA were related to patients with other underlying diseases, elderly patients and surgical procedures (see also 2.6.3). In general, risk factors for hospitalized patients to contract a MRSA infection are prolonged hospitalization, care in an intensive care unit, prolonged antimicrobial therapy, surgical procedures, and close proximity to a patient in the hospital who is infected or colonized with MRSA (Salgado et al., 2003).

ESBL-producing *Escherichia coli*

ESBL-producing *Escherichia coli* carriers and patients are associated with exposure in the community and in health care. In the community they may cause urinary tract infections. Influx from the community to the hospital may result in wound and invasive infections in immunocompromised patients.

Risk groups for carriage

The prevalence of ESBL-producing *E. coli* is increasing worldwide in health care and in the community. This increase is related to pandemic spread of *Escherichia coli* O25 ST131, harbouring an FII-plasmid with CTX-M-15 and plasmid addition systems (Coque et al., 2008). In the Netherlands, this increase may be partly related to exposure to ESBL-producing strains from the food chain or environmental sources (Leverstein-Van Hall, 2009). Faecal carriage of ESBL-positive *E. coli* was not found in Dutch patients with relapsing UTI (Stobberingh, personal communication). Faecal carriage among Dutch broiler farmers occurs frequently. In a pilot study in the faecal flora of 18 farmers, 6 were found positive for ESBL-producers in their faeces (personal communication Mevius). Several studies in other countries revealed that prevalences of ESBL positive *E. coli* among healthy individuals are in the range 1.4-12.2% and 5.5-14.1% in outpatients (Castillo García et al., 2007; Kader et al., 2007; Mesa et al., 2006b; Moubareck et al., 2005; Munday et al., 2004; Pallecchi et al., 2007; Valverde et al., 2004). In most studies at least 50% of the isolates are CTX-M producers. Recent use of antimicrobials and being a relative of a patient with UTI (Rodríguez-Baño et al., 2008a; Tian et al., 2008) increases the risk for carriage in the community. Other risk factors found were: age >60 years, coexisting conditions as measured by the chronic disease score, in-hospital use of piperacillin-tazobactam and vancomycin (Harris et al., 2007), low gestational age or low birth weight and exposure to third-generation cephalosporins (Linkin et al., 2004).

Risk groups for infection in the community

In the Netherlands, approximately 1% of the UTI in general practice patients are caused by ESBL-producing *E. coli*, which is approximately 5400 cases annually (Stobberingh,

personal communication); risk factors have not been determined. Risk factors found in other countries were: advancing age, female gender, other underlying medical conditions, foreign travel, previous invasive procedures of the urinary tract, follow-up in outpatient clinic, and recent use of aminopenicillins, cephalosporins, and fluoroquinolones (Apisarnthanarak et al.2007; Calbo et al., 2006; Laupland et al., 2008; Moor et al., 2008; Rodríguez-Baño et al., 2008b).

Risk groups for infection in health care institutions

In the Netherlands, the overall frequency of ESBL-producing *E. coli* in hospital isolates has increased from less than 1% in 1999 (Stobberingh et al., 1999) to 2.6% in 2004 (Bouchillon et al., 2004) and 6% in 2006 (Mouton, 2007). The prevalence of ESBL-positive *E. coli* bloodstream infections has rapidly increased from <1% in 2000 to 4.2% in 2008 (EARRS, 2009). Besides hospital patients, in France also patients in long-term-care facilities were found to be colonized or infected with ESBL-positive *E. coli* (Nicolas-Chanoine and Jarlier, 2008); the Dutch situation is under investigation.

Risk factors frequently associated with ESBL *E. coli* bloodstream infections are: prior use of beta-lactam antibiotics or fluoroquinolones, living in a residential care home, urinary catheterization or other invasive procedures, length of hospital stay, severe underlying disease, prior admission to the intensive care unit or previous extended hospital stay (Du et al., 2002; Ho et al., 2002; Peña et al., 2006; Rodríguez-Baño et al., 2008b; Skippen et al., 2006).

ESBL-producing *Klebsiella* spp.

ESBL-producing *Klebsiella* spp. carriers and patients are mainly associated with exposure in health care settings.

Risk groups for carriage

The carrier rates of ESBL-producing *Klebsiella* spp. in the Netherlands are unknown. Reported carrier rates of *Klebsiella* spp. in the community are 1-6% and 5-38%, for the intestinal tract and nasopharynx respectively. Carrier rates in the hospital environment are elevated up to 77% in stool samples of patients and increases with the length of stay. In the USA, faecal carrier rates of ESBL-producing *Klebsiella* spp found were 26% of the inpatients, 15% of the outpatients, and 13% of the healthy individuals (Podschun and Ullmann, 1998). Risk factors found for faecal carriage of ESBL-producing *K. pneumoniae* at ICUs were clinical severity score at admission, arterial catheterization, total parenteral nutrition, urinary catheterization, mechanical ventilation, and previous antibiotic therapy. Duration of urinary catheterization and mechanical ventilation were also found as independent risk factors (Peña et al., 1997).

Risk groups for infection in the community

Klebsiella spp. may cause community-acquired pneumonia and liver abscesses, which is associated with chronic alcohol use and mainly occurs in Africa and Asia (Ko et al., 2002).

Risk factors for infection with an ESBL-producing organisms in non-hospitalized patients were recent antibiotic use, residence in a longterm care facility, recent hospitalization, age ≥65 years, and male sex (Ben-Ami et al., 2009). Nursing homes residents are also more at risk of infections with ESBL-producing *Klebsiella* (Goldstein et al., 1993) and may bring these bacteria into the hospital setting (Paterson and Bonomo, 2005).

Risk groups for infection in health care institutions

Klebsiella spp. infections in health care institutions primarily occur in immunocompromised patients with underlying diseases and prematures (Podschun and Ullmann, 1998). In general, patients at high risk for developing infection and colonization with ESBL-producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endotracheal tubes, central venous lines) for a prolonged duration. Antibiotic use is also frequently found as a risk factor for ESBL-producing bacteria (Paterson and Bonomo, 2005).

2.6.3 Type and severity of adverse health consequences

Type of health consequences

The type and severity of the health consequences resulting from infections with antimicrobial-resistant bacteria may be affected by pathogen, host and treatment factors (Cosgrove, 2006; Maragakis et al., 2008). It is assumed that antimicrobial-resistant bacteria cause similar clinical pictures as susceptible bacteria, since evidence is lacking that antimicrobial-resistant bacteria are more virulent than susceptible bacteria, with the possible exception of community-associated MRSA (Cosgrove, 2006; Martínez-Aguilar et al., 2004). Below a summary of the main health consequences are summarized for each of the example bacteria.

Severity of health consequences

Although similar clinical pictures in antimicrobial-resistant bacteria are seen, the overall outcomes of infections with antimicrobial-resistant bacteria may be more severe than the outcomes of infections with antimicrobial susceptible bacteria (Carmeli et al., 2002; Lee et al., 2006). These differences may be explained by host related factors (e.g. underlying illness) or treatment factors. Treatment factors include delay in the administration of appropriate antimicrobial therapy, the requirement of more toxic or less effective antimicrobials, or, in case of resistance to all available antimicrobials, requirement of surgical

procedures (Cosgrove, 2006). Differences found in morbidity and mortality between susceptible and resistant bacteria will be discussed in section 2.7.1 (Disease burden).

Quinolone-resistant *Campylobacter*

Campylobacter primarily causes acute gastroenteritis that usually is self-limiting and in most cases lasts less than a week. Complications are rare, but the following complications may occur: reactive arthritis, irritable bowel syndrome, inflammatory bowel disease and Guillain-Barré syndrome (Haagsma et al., 2010; Havelaar, 2007). There are no indications that illness related to quinolone-resistant *Campylobacter* differs from susceptible *Campylobacter* (Wassenaar et al., 2007). Treatment of gastroenteritis caused by C. jejuni with antimicrobials is only indicated for patients with high or persistent fever, dysentery or immunocompromised hosts (Bos et al., 2006). Quinolones are excluded for empiric treatment of gastroenteritis treatment, hence treatment options are reduced.

LA-MRSA

In general, *Staphylococcus aureus* may cause various primary and secondary infections from superficial infections to systemic infections and toxin related disease (see Balows, 1991; www.rivm.nl). LA-MRSA is a relatively new strain and its importance for human health is still under investigation. Although the majority of infections seem to be skin or soft tissue infections, this strain is capable of causing serious infections in humans (Van Belkum et al., 2008; Wassenberg et al., 2008). Clinical cases of LA-MRSA published concerned a young mother with mastitis living on a pig farm (Huijsdens et al., 2006), a 63 year old woman with a kidney transplant with endocarditis (Ekkelenkamp et al., 2006), hospital acquired ventilator-associated pneumonia (Witte et al., 2007), soft tissue infection of a wound resulting from a pig bite (Declercq et al., 2008), lung and wound infections in elderly patients (Yu et al., 2008), skin and soft tissue infections, sinusitis, and a severe invasive infection with multiple organ failure after knee surgery (Lewis et al., 2008). LA-MRSA isolates were found to be negative for the exfoliative toxin genes, the leukotoxin genes, and toxic shock syndrome gene (Huijsdens et al., 2006; Van Duijkeren et al., 2008), making toxin related disease (gastroenteritis, toxic shock syndrome) unlikely. Panton-Valentine leukocidin (PVL) genes are rarely found in LA-MRSA (Van Belkum et al., 2008; Van Loo et al., 2007), which means that associations with severe necrotizing pneumonia which is typical for community acquired MRSA (CA-MRSA) are unlikely. LA-MRSA is a multidrug resistant bacterium; treatment options are strongly reduced.

ESBL-producing *Escherichia coli* and *Klebsiella* spp.

Escherichia coli and *Klebsiella* spp. are opportunistic pathogens that cause a wide range of infections, such as wound infections, gastroenteritis, urinary tract infections, pneumonia, meningitis and bacteraemia (Ko et al., 2002; Podschun and Ullmann, 1998). ESBL-producing *E. coli* is an agent of urinary tract infections in the community, but is also associated with invasive infections. ESBL-producing *Klebsiella* spp. is an important cause of nosocomial infections of the respiratory tract, intra-abdominal infections and bacteraemia. Community-acquired pneumonia and liver abscesses mainly occur in Africa and Asia (Ko et al., 2002). An alarming trend of associated resistance to other classes of antimicrobials has been observed in ESBL-producing bacteria in both the community and health care settings. ESBLs are an important reason for initial therapy failure with cephalosporins, which has serious implications for infection control (Pitout and Laupland, 2008). When the resistance percentage rises above 5%, the use of ceftriaxon in the empiric treatment of invasive *E. coli* infections is not longer possible.

2.7 Magnitude of the problem

The transmission of antimicrobial resistance from food animals to humans may have serious consequences that affect society through increased disease burden, economic costs, and social consequences. The determination of the present and the future magnitude of these consequences as well as the risk perception of the general public are important issues when setting policy priorities. In this chapter, data on disease burden (2.7.1), economic consequences (2.7.2), and social consequences and risk perception (2.7.3) are summarized.

2.7.1 Disease burden

Data on the disease burden caused by antimicrobial-resistant bacteria in the Netherlands is scarce and also internationally the magnitude of the impact of antimicrobial resistance on human health outcomes remains largely unknown (Maragakis et al., 2008). Disease burden is usually expressed in Disability Adjusted Life Years (DALY); this is a composite measure developed by the World Health Organization (WHO) that combines morbidity and mortality (Murray, 1996). Antimicrobial resistance may increase disease burden by increased incidence of disease, increased morbidity, and/or increased mortality. For infections caused by antimicrobial-resistant bacteria the majority of studies therefore compare health outcomes of patients infected with resistant strains to a control group of patients with susceptible strains or sometimes without infection, in order to determine the incremental disease burden. Below disease burden methodology is briefly explained, data needs are identified, and available data on the disease burden of the

selected hazards in the Netherlands are summarized.

Disease burden methodology

One DALY is one lost year of healthy life; the number of DALY that is lost due to a pathogen in a human population is the total number of years lost due to premature deaths in this population (Havelaar, 2007) plus the total number of years that patients have spent with disease (YLD), weighed for the loss of quality of life. All relevant health outcomes that a pathogen may cause in a specified human population should be taken into account (Havelaar, 2007). Incremental disease burden is usually calculated in case-control studies and is often expressed in increased risk of death or attributable length of stay (LOS). When calculating the incremental disease burden, there are several methodological issues that need to be considered, such as the selection of the control group and confounding factors, such as comorbidity (Maragakis et al., 2008).

Data needs

For calculation of disease burden in DALY, first qualitative information on relevant health outcomes caused by the pathogen and their underlying relations is needed, which can be depicted in an outcome tree. Next, quantitative information on all relevant health outcomes needs to be collected. To quantify YLL, the total number of fatal cases due to each health outcome, the age distribution of the fatal cases and a life expectancy table is needed. For YLD the disease duration of each health outcome, disability weights that indicate the severity of the health outcomes, and the total number of cases with the health outcomes need to be collected. To calculate the incremental disease burden due to resistance, data on the abovementioned parameters for patients with infections due to resistant bacteria and a matching control group is needed.

Quinolone-resistant *Campylobacter*

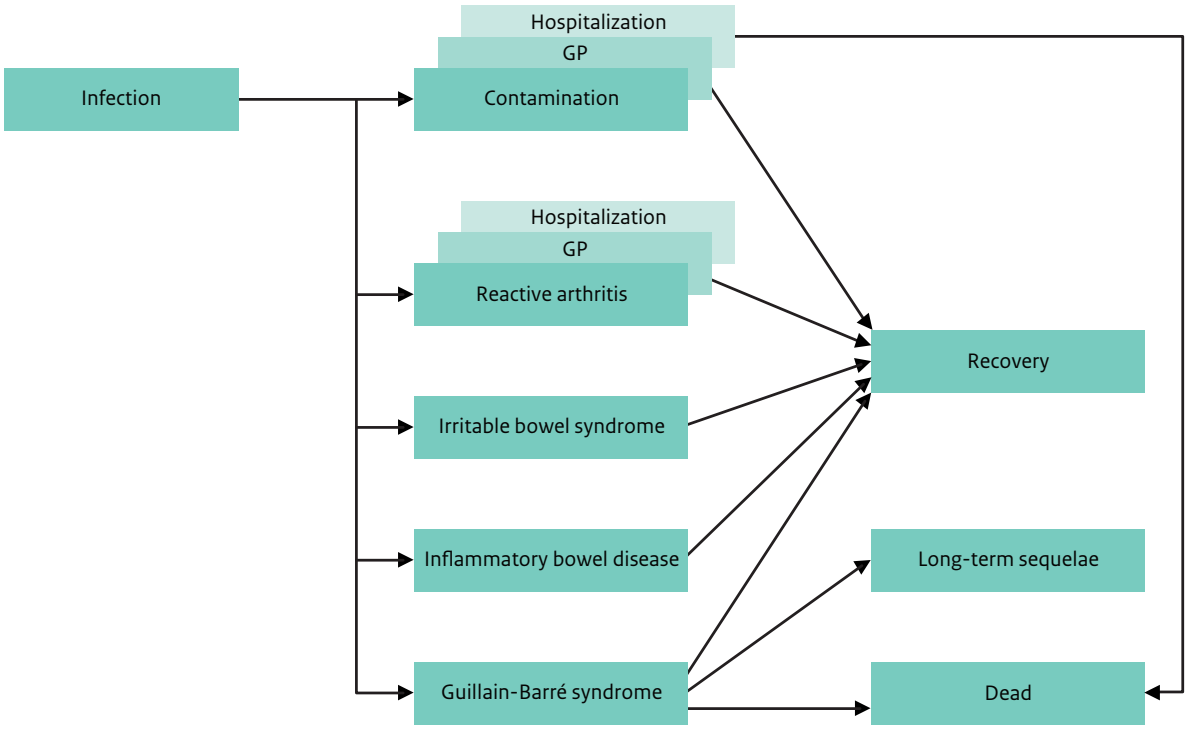
The total number of symptomatic *Campylobacter* infections in the Netherlands is estimated to be 79,000 (Haagsma et al., 2009). In 2007, 6700 laboratory confirmed cases of campylobacteriosis were reported, of which the majority (90%) was caused by *C. jejuni*. These laboratory-confirmed cases represent the more severe cases of gastroenteritis of which an estimated 30% (Van Pelt, personal communication) are likely to be treated with antibiotics; exact figures on antimicrobial prescription are missing. Treatment of gastroenteritis caused by *C. jejuni* with antimicrobials is not indicated unless patients suffer from high or persistent fever, dysentery or are immunocompromised (Bos et al., 2006). Approximately 25% of the laboratory cases were admitted to the hospital (Van Pelt et al., 2008a). Mortality is mainly related to gastroenteritis (approximately 25 cases per year) and Guillain-Barré syndrome (approximately 2 cases per year), (Kemmeren et al., 2006). The quinolone resistance rate in clinical isolates was found to be 50% (MARAN-2008).

There are no indications that the relevant health outcomes as described in section 2.6.3 are different for quinolone-resistant *Campylobacter*, hence, a similar outcome tree as for susceptible *Campylobacter* can be used (Figure 15).
A few studies from Denmark, UK, USA and Australia have investigated differences in type and severity of adverse health effects, particularly duration of diarrhoea between infections with quinolone-resistant and susceptible *Campylobacter* (Smith et al., 1999; Painter et al., 2002; Engberg et al., 2004; Nelson et al., 2004; Unicomb et al., 2006). These studies presented conflicting findings and after reanalysis Wassenaar et al. (2007) concluded that there are no indications that infections with resistant and susceptible strains differ in severity. Helms (2005) studied invasive illness and mortality due to infections with quinolone-resistant *Campylobacter* strains in Denmark. They conclude that there is a 6-fold increased risk of an adverse event within 30 days in patients infected with quinolone-resistant *Campylobacter* strains compared to cases infected with susceptible strains. However, it has to be noted that invasive disease is rare and that these results mainly concerned patients with underlying diseases and elderly patients (Wassenaar et al., 2007).
Given the analysis by Wassenaar, (MARAN-2007) and the exclusion of quinolones for the empiric treatment of gastroenteritis (Bos et al., 2006), there are no indications that the disease burden of gastroenteritis caused by *Campylobacter* has increased as a consequence of quinolone resistance in the Netherlands.
There is no information available on quinolone resistance in *Campylobacter* and the occurrence of extra-intestinal disease as these health outcomes are sequels of gastroenteritis and appear some time after infection at which the bacterium often has disappeared and resistance cannot longer be determined. The extra-intestinal health outcomes are not treated with antibiotics; it is assumed that quinolone resistance does not increase the disease burden of these health outcomes.
It is therefore concluded that there are no indications that the disease burden has increased as a consequence of quinolone resistance. The overall disease burden is therefore estimated at 50% of the total disease burden of *Campylobacter*, i.e. approximately 40,000 symptomatic infections of which over 800 patients are hospitalized and approximatey 15 fatal cases will occur, resulting in 1400-1600 DALY (Haagsma et al., 2009). The individual disease burden is assumed to be similar, i.e. 0.018-0.023 DALY per case (range depending on discounting), (Haagsma et al., 2009). Approximately 50% of the disease burden consists of YLD due to extra-intestinal disease (Haagsma et al., 2009).

LA-MRSA

In 2009, 21,546 human clinical isolates of *Staphylococcus aureus* were registered in the Dutch Infectious diseases

Figure 15 Outcome tree for quinolone-resistant *Campylobacter jejuni* (J. Haagsma, personal communication).



Surveillance Information System for Antibiotic Resistance (ISIS-AR). Approximately 1.5% of these isolates were found to be resistant to beta-lactams and hence are presumed MRSA isolates. ISIS-AR is assumed to represent a subsample of approximately one third of the Dutch medical microbiological laboratories. Extrapolation of these numbers results in an estimated total of approximately 60,000 clinical isolates of *S. aureus* that were submitted for laboratory testing. The Dutch medical microbiological laboratories submit the MRSA isolates to the MRSA surveillance (RIVM) for further analysis. The MRSA surveillance showed that, in recent years, approximately 500-900 MRSA and approximately 100 LA-MRSA clinical isolates are tested annually (Haenen et al., 2009; Haenen et al., 2010). It is unknown which part of the MSSA isolates is livestock-associated.

The majority of relevant health outcomes of LA-MRSA as described in section 2.6.1 seem to be similar to HA-MRSA infections. Until now there are no fatal LA-MRSA cases known. There are no indications that there are differences in the proportion of superficial and invasive infections between LA-MRSA and HA-MRSA (Haenen et al., 2009) and LOS was not found to be significantly different (Wassenberg et al., 2010). It is therefore concluded that there are currently no indications that the individual disease burden of LA-MRSA infections differs from HA-MRSA infections.

In international studies, MRSA infections are associated with an increased LOS and a higher mortality as compared to MSSA infections due to initial therapy failure (Cosgrove, 2006). The MRSA disease burden has neither been quantified in the Netherlands, nor in other countries. In 2007, approximately 10% of all reported MRSA infections were caused by LA-MRSA (Haenen et al., 2009); this percentage has increased to almost 16% in 2008 due to descreasing numbers of non-livestock-associated MRSA (Haenen et al., 2010)1. LA-MRSA infections concern patients that are exposed to a new reservoir of MRSA, i.e. food animals. The overall MRSA disease burden has therefore increased with the rise of LA-MRSA infections.

ESBL-producing bacteria

In 2009, 56,345 and 8264 human clinical isolates of *E. coli* and *Klebsiella* (*Klebsiella oxytoca* and *K. pneumoniae*) were registered in ISIS-AR. Approximately 3.4 and 4.3% respectively were found to be resistant to third generation cephalosporins and hence presumed ESBL-producing isolates. Extrapolation of these numbers results in an estimated total of approximately 160,000 ESBL-negative and 5700 ESBL-positive *E. coli* and approximately 23,000 ESBL-negative and 1000 ESBL-positive *Klebsiella* human clinical isolates that were submitted for laboratory testing. It is unknown which part of these isolates is livestock-associated.

Based on ISIS-AR data, there were an estimated 500 invasive infections by ESBL-producing bacteria reported in 2009 (Leverstein-van Hall, personal communication). In general practice patients, an estimated 1% of the UTI

(approximately 5400 cases) were caused by ESBL-producing *E. coli* (Stobberingh, personal communication). Infections with ESBL-producing bacteria are associated with increased length of hospital stay, increased morbidity and mortality (Maragakis et al., 2008). The disease burden has not yet been quantified, but, given the high number of invasive infections, it is likely to be substantial. With the increase of infections caused by ESBL-producing bacteria, the disease burden of infections caused by ESBL-producing bacteria has increased.

2.7.2 Economic consequences

There is little information available on the costs of antimicrobial resistance in the Netherlands and also internationally, the magnitude of its impact on economic outcomes remains largely unknown and is the subject of ongoing investigation (Maragakis et al., 2008). In general, the estimated costs of resistance are considerable. (Roberts et al., 2009) for example, calculated that the medical costs attributable to infections with antimicrobial-resistant organisms in high-risk hospitalized adult patients in the USA range from $18,588 to $29,069 per patient and ranged from $10.7-$15.0 million for society. Most studies only consider the hospital perspective, in particular the estimated costs of HA-MRSA for health care, both in the Netherlands (Gezondheidsraad, 2006; Nulens et al., 2008; Vriens et al., 2002) as abroad (Gould, 2006). In order to understand the full economic effect of antimicrobial resistance, the societal perspective should be investigated, including the indirect and long-term consequences, such as decreased effectiveness of antimicrobial drugs. An overview of the various costs and perspectives are given in Maragakis (MARAN-2008). Similar to disease burden, healthcare costs of resistance are often calculated as excess costs, i.e. compared to the costs of susceptible strains, e.g. costs of excess morbidity (e.g. longer hospital stay) and excess mortality. Below a framework for calculating the costs to society is presented, data needs are identified, and available data on the selected hazards in the Netherlands are summarized.

Costs of resistance framework

The cost of antimicrobial resistance to society may be calculated with a similar framework as the cost of illness to society and should take into account the specific costs involved in the detection and isolation of carriers. Cost of illness methodology is described in Kemmeren et al., 2006 and is based on Oostenbrink, 2004. Briefly, cost of illness (COI) consists of direct health care costs (DHC), direct non-health care costs (DNHC), indirect health-care costs (IHC), and indirect non-health care costs (INHC). DHC are all costs that are directly connected to prevention, diagnostics, therapy, revalidation and the care of patients. For antimicrobial resistance, DHC should also include the costs of screening of risk groups, costs due to isolation of patients found positive, costs involved in non-activity of staff tested positive and outbreak costs, such as loss of hospitalization days and surgeries in case of closing of wards. Potential DHC of resistance are the costs involved with changing empiric therapy and limited therapy choice after diagnosis when resistance levels increase over a certain threshold. DNHC are e.g. the time and travel costs that patients make, but also include costs of constructional adaptations to the patient's house, co-payments and informal care. Indirect costs are costs that arise as a secondary consequence of the illness or treatment. IHC are the future savings on health care costs due to premature death and, in accordance with Oostenbrink, 2004, are usually not considered. INHC comprises the value of production lost to society due to disease as a consequence of temporary absence from work, permanent or long-term disability, and premature mortality, but also legal costs and expenses for special education.

Data needs

In general, to calculate the COI one needs to find out the volumes of use of resources and the actual economic costs of each of these items. Moreover, the number of cases per age group is needed to calculate costs of e.g. sickness leave (INHC). To calculate long-term consequences, estimates on future precaution measurements, future empiric treatment, long-term effects on health, labour, etcetera, need to be assessed. To calculate excess costs for health care, data on incremental disease burden characteristics, such as excess LOS and mortality need to be collected.

Quinolone-resistant *Campylobacter*

Since no indications were found that the disease burden caused by *Campylobacter* has increased as a consequence of quinolone resistance and no additional isolation and screening measures are needed due to the absence of person-to-person spread, it is assumed that the COI of fluoroquinolone-resistant *Campylobacter* is similar to that of susceptible *Campylobacter*. The COI of *Campylobacter* is estimated to be €27.4 million, which is €347 euro per case (Haagsma et al., 2009). The majority of costs are INHC (approximately €15 million) and DHC (approximately €12 million).

LA-MRSA

The COI of LA-MRSA has not been quantified. However, the search-and-destroy policy is also applied to LA-MRSA, which includes screening of risk groups, post exposure screening of contacts and isolation treatment of patients found positive. There has been a strong increase in MRSA screenings and in the number of detected carriers due to LA-MRSA. For example, after the inclusion of patients in direct contact with pigs and veal calves as a risk group for MRSA screening, a 15% increase in screenings, including 24 post-exposure screening, in

39 hospitals were observed in juli-december 2006 (Wassenberg et al., 2008). This resulted in a 44% increase of detected carriers, which obviously has led to an increase in DHC. The number of LA-MRSA contact infections is relatively low, which reduces the costs of hospital outbreaks, which for HA-MRSA are reported to be high (Nulens et al., 2008; Vriens et al., 2002).

ESBL-producing bacteria

The COI of ESBL-producing bacteria has not been quantified; however, the economic consequences due to resistance are likely to be substantial. Initial treatment of infections with ESBL-producing bacteria may fail resulting in delayed treatment, increased disease burden, and hence increased COI. Alternative treatment options for these multidrug resistant bacteria are limited, more expensive and may require hospitalization. Moreover, for ESBL-producing bacteria measures for multidrug-resistant micro-organisms(BRMO) (WIP, 2009) are applied, which includes screening of risk patients, i.e. patients at the ICU and patients from foreign hospitals, and the treatment of positive patients in contact isolation. An increasing number of carriers will therefore lead to an increasing DHC.

2.7.3 Social consequences and risk perception

Social consequences

The impact of antimicrobial resistance on everyday life of people may be severe in individual cases, but has not been studied thoroughly. Studies on MRSA patients and carriers in healthcare settings indicate that, besides somatic problems, they may in particular experience psychological problems, such as depression, anxiety, feeling stigmatized or socially isolated and may have less social contacts due to the fear of contaminating people in their daily environment (Donaldson et al., 2007; Hendrickx et al., 2009; Verhoeven et al., 2009). Social consequences for patients infected with quinolone-resistant *Campylobacter* and ESBL-producing bacteria have not been documented.

Risk perception

Risk perception of the general public has become an important issue to policy makers as it reflects the subjective assessment that people make of the probability that a negative event will happen and how concerned they are with the consequences. Risk perception of the general public on antimicrobial resistance from food animals has not been studied, but given the recent commotion in the various media on this LA-MRSA and ESBL-producing bacteria, it is presumed that people are aware of the contribution of food animals to antimicrobial resistance in humans and see it as a serious cause for concern. This topic needs more attention, so the risk communication can be tailored to the needs of the general public. For this purpose, the experience gained in the investigation of risk perception of food safety (De Jonge et al., 2010) and environmental issues may be of use.

3 Antimicrobial usage and resistance in (food-producing) animals and the environment

3.1 Usage in food animals

Introduction

The use of veterinary medicines may lead to risks concerning human health, environment, food safety, animal health and animal welfare. The extent to which antibiotics are used for veterinary purposes in food animals is an important determinant for the development of antibiotic resistance within the treated animal populations. It is important to have proper insight in the use of antimicrobials for the treatment of (food-producing) animals. This chapter contains information about the monitoring results in the Netherlands as well as on antibiotic use in other countries. The Dutch data in this

report are based on the information collected in the Farm accountancy Data Network of the Agricultural Economics Institute (LEI), as well as data from the Association of manufacturers and importers of veterinary medicines (FIDIN). Data on antibiotic use in other countries are mainly based on publicly available reports that are reported by various European countries.

Critically important antibiotics in Veterinary medicine

Parallel to the list of critically important antimicrobials in human medicine of the WHO, the OIE has developed a list of critically important antimicrobials in veterinary medicine. The objective of this list is described as follows: 'The overlap of critical lists for human and veterinary medicine can provide further information, allowing an appropriate balance to be struck between animal health needs and public health considerations'(FAO/OIE/WHO, 2004). Antimicrobials were considered to be veterinary critically important drugs when a majority of the OIE member states had identified their importance *and* antimicrobials within this class were considered essential against specific infections with a lack of sufficient therapeutic alternatives. Antimicrobials were categorized as Veterinary Highly Important Antimicrobials when only one of the two criteria were met (that is either considered critically important by a majority of the member states or regarded as essential for the treatment of specific diseases without equivalent alternatives). The remaining drugs were categorized as veterinary important. In table 4 the antimicrobial classes are shown as categorised by the OIE. The original list is available from the OIE website (http://www.oie.int/downld/Antimicrobials/OIE_list_antimicrobials.pdf).

Table 4 Categorisation by the OIE of veterinary important antimicrobials for food-animals

Veterinary Critically Important	aminoglycosides
	cephalosporins
	macrolides
	penicillins
	phenicols
	quinolones
	sulfonamides
	tetracyclins
Veterinary Highly Important	rifamycins
	fosfomycinionophores
	lincosamides
	pleuromutilins
	polypeptides
Veterinary Important	bicyclomycin
	fusidic acid
	novobiocin
	orthosomycins
	quinoxalines
	streptogramins

Technical units for the quantification of antibiotic use: use in kilograms and daily dosages per animal year

Antibiotic use can be quantified in various ways. Usage can be expressed in total weight (kg) or units of the active ingredients, data which are fairly easy to obtain. In the Netherlands the FIDIN (vereniging van Fabrikanten en Importeurs van Diergeneesmiddelen) publishes a yearly overview, based on the total amount of antibiotics sold in our country. According to FIDIN, data are obtained that cover 98% of the Dutch sales market.
These data are readily available and are very useful for a general impression of the usage in animal husbandry. A major drawback is that the figures can not be further specified, other than the route of admission (i.e. oral or systemic use). Therefore no information is available on the animal species the antibiotics are used for or the type/category of husbandry (i.e. fattening or production animals), etcetera.

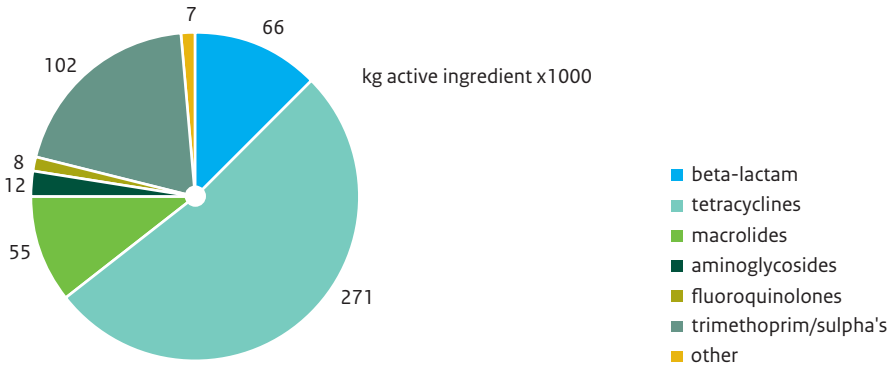
For the monitoring in public health, antibiotic use is expressed in DDD per 1000 inhabitants per day (primary health) or either as DDD per 100 patient-days or DDD per 100 admitted patients (hospital use), see also section 2.5.2. In veterinary medicine, similar units of measurement are being used.
In the Netherlands, the pharmacy of the Veterinary Faculty has developed the defined Animal Daily Dose (ADD). This can be defined as the assumed maintenance dose per day for a drug for its main indication in a specific animal species. This dosage has been determined for a standard animal, that is an animal with an average weight in a specified age group. Also in other countries such units of measurement have been developed, for instance in Denmark (Jensen et al., 2004a) and Belgium (Timmerman et al., 2006). Unfortunately this has as yet not led to an international agreed upon unit of ADD (or DDD_{animal}), which is important for accurate comparison of antibiotic use between different countries.

Antibiotic use in the Netherlands

Monitoring by FIDIN

In 2008 a total of 521 tons of active ingredient have been sold in the Netherlands (FIDIN, 2009). Data are reported at the level of pharmacotherapeutic groups (groups of active ingredients, such as tetracyclines and quinolones). The data do not provide insight into the use per animal species or type of animal (i.e. veal calves versus dairy cows, broilers versus laying hens or piglets versus sows or fattening pigs).
Figure 16 shows the relative amount of antibiotics of different antimicrobial classes sold in 2008 in the Netherlands. Tetracyclines are the most widely used

Figure 16 Sales of antibiotics in the Netherlands in 2008. Source: FIDIN-antibiotic report 2008 (FIDIN, 2009).



antibiotics in veterinary medicine (52% of the total weight of antibiotics sold in the Netherlands in 2008). Other groups of antibiotics that are commonly used are trimethoprim/sulpha's (20% of the total use), beta-lactam antibiotics (13%) and macrolides (11%). Approximately 90% of the use encompasses antibiotics registered for oral use like premix, topdressing and medication in drinking water and is used to treat animal groups rather than individual animals.

Monitoring by LEI

On request of the Ministry of Economic Affairs, Agriculture and Innovation (EL&I), LEI provides yearly analyses on the use of antibiotics on Dutch livestock farms. The antibiotic use in the various animal production sectors are monitored continuously and in great detail to provide insight in the underlying factors and determinants for veterinary antibiotic use. This is based by detailed monitoring of a stratified sample of Dutch farms that are part of the LEI's Farm Accountancy Data Network (FADN). Data on the veterinary use of antibiotics is reported on a yearly basis in MARAN.

Figure 17 shows the antibiotic use at a sample of sentinel farms in 2007. The four vertical lines in the figure indicate the confidence interval, i.e. on the basis of this sample the average antibiotic use in the Netherlands can be stated to lie within the upper and lower limits with 95% confidence (MARAN-2007).

Trends in antibiotic use in the last decade

The total use of antibiotics (both as growth promoters and for therapeutic use) has remained relatively stable over the past 10 years despite the banning of growth promoters (2006) and a decrease in the total number of livestock animals (Figure 18). However, therapeutic usage of antibiotics in food animals has been steadily increasing over the years (Figure 18 and 19). A total amount of 322 tons of active ingredient were consumed in 1999 to 521 tons in 2008, an increase of 62%.This accounts for an average increase of almost 7% per year (FIDIN, 2009). The data of 2008 have shown a decrease of 12% compared to the usage in 2007 (590 tons), but it is estimated by the FIDIN that at least half of the decrease was the result of stockpiling by the end of 2007 with an actual decrease of 7% (instead of 12%) to be more likely. It is stated that a possible explanation for the decrease of sales data in 2008 compared to preceding years might be an increased attention and awareness of the risks of antibiotic resistance in the animal production (FIDIN, 2009).
When sales data in the past decade are related to livestock production data (in kg live weight) an increase from 0.13 mg antibiotics/kg live weight in 1999 to 0.23 in 2008 is observed (Figure 20). Also antibiotic use in DDD per average animal per year shows a rising trend, from the administration of a daily dosage of antibiotics on

Figure 17 Average number of daily dosages per animal year in 2007, with the corresponding 95% confidence intervals (MARAN-2007).

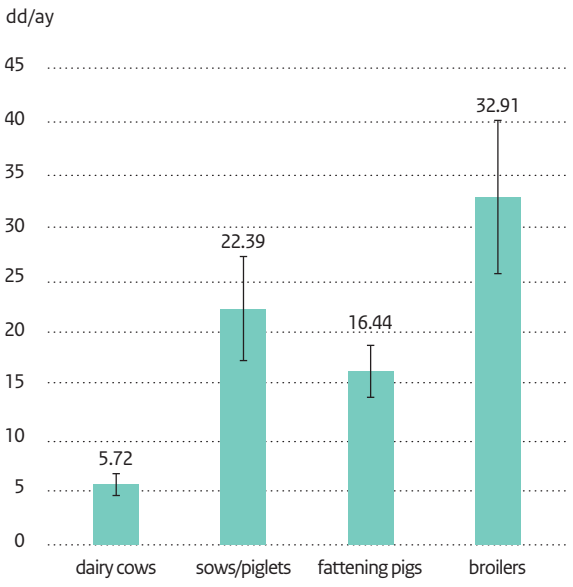


Figure 18 Veterinary antibiotic use from 1999-2008 based on FIDIN data (MARAN-2008).

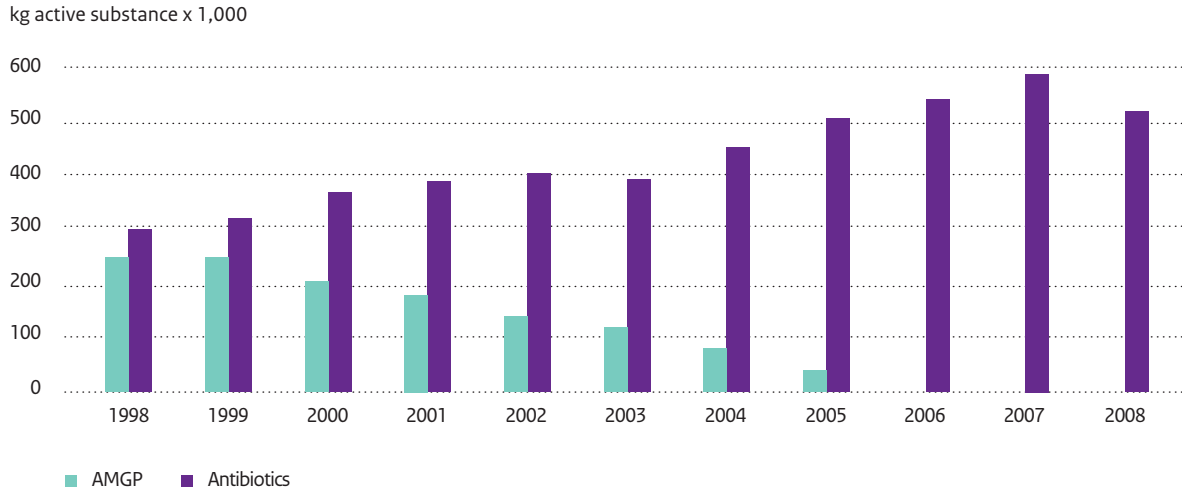


Figure 19 Veterinary therapeutic use in the Netherlands, 1999 – 2008 by antibiotic classes, based on FIDIN data (MARAN-2008).

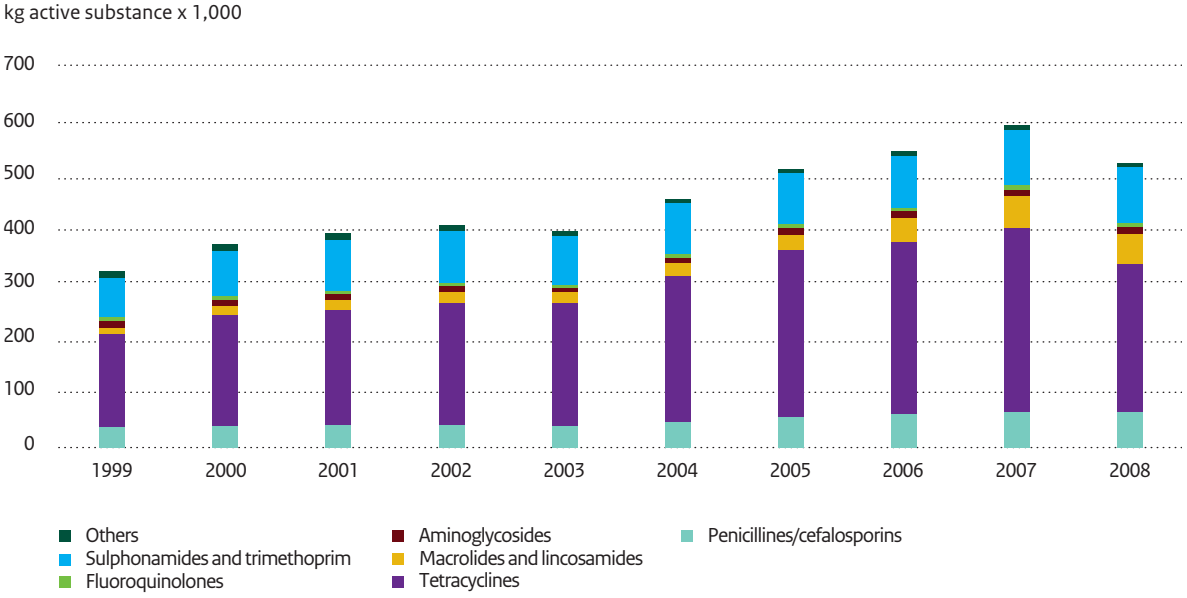


Figure 20 Total therapeutic antibiotic use 1999-2008, in mg per kg live weight (MARAN-2008).

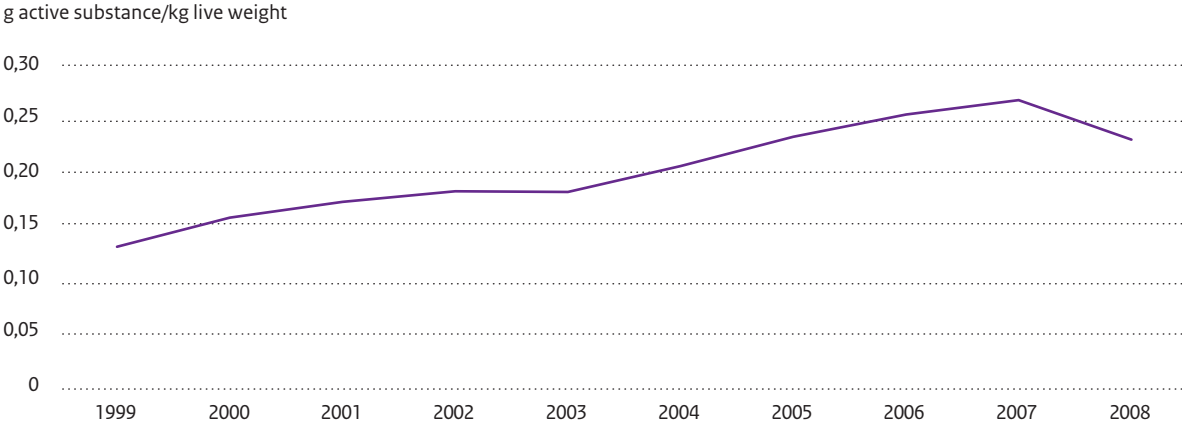


Figure 21 Tendencies in relative antibiotic usage from 2004-2008 in percentages daily dosages per animal year at the sentinel farms in 2004 – 2008 (daily dosages per animal year in 2007 = 100%). For veal calves only data from 2007 and 2008 are available (MARAN-2008).

approximately 20 days in 2001 to 30 days in 2007 for an average animal in the Netherlands (Van Geijlswijk, 2009). Figure 21 shows the changes in the use at all farm categories in the sentinel farms during the years 2004-2008. The outcome of the calculations is indexed, using 2007 as baseline year. The continuous line represents the calculated average use. The 95% confidence intervals, calculated as from 2005 (indicating that with 95% certainty, the average antibiotic use on a national level, expressed in terms of the number of daily dosages per animal year, will lie within the upper and lower limits) are indicated by the dotted lines shown in Figure 21. The antibiotic use for fattening pigs and, in particular, broilers, exhibits an evident increase (MARAN-2008).

The increase in usage in the past decade has likely been powered by the ban of the growth promoters and the upscaling of farm sizes. The use of antibiotics is a cheap (and often effective) solution when dealing with health problems that are associated with intensive husbandry and control on inappropriate usage is lacking or insufficiently effective. The continuous increase in antibiotic usage and the simultaneous tendency of increasing levels of resistance in animal bacteria has led to growing concerns about the negative effects and public health risks of antibiotic use in animal husbandry, both by the public as by the animal sectors. This has resulted in the developments of covenants by the end of 2008 aimed at the reduction of antibiotic resistance, see also section 6.1.

Antibiotic use in other countries

Besides information about the monitoring results in the Netherlands, also antibiotic use data from other countries are available. These are mostly based on public reports from a number of European countries.

Although an accurate comparison requires more sophisticated data (for instance on the type or intensity of animal husbandry and relative use of active ingredients) it is obvious that compared to other countries from which data are available the antibiotic consumption for veterinary purposes in the Netherlands is relatively high (Figure 22).

Generally perceived explanations for this outlying position are the intensity of the animal industry in the Netherlands and different antibiotic preferences compared to other countries.

An overview of European antibiotic use by group of medicines gives an insight into the veterinary antibiotics policy pursued in the various countries (see Figure 23).

The following remarks can be made about the groups of medicines used in the various countries:

A distinction can be made between three general treatment strategies in Europe:

- Scandinavian countries: a strategy primarily based on beta-lactam antibiotics
- Denmark: a strategy based on tetracyclines + macrolides + beta-lactams
- Other European countries: a strategy primarily based on tetracyclines

Norway uses relatively large amounts of fluoroquinolones (15%) in the fish-breeding sector, which is more than the amount administered to humans in the Netherlands.

All countries use roughly the same amount of trim/sulpha combinations (approximately 8%).

The above comparison has been made for all years from 2001 to 2006 inclusive, but holds for the years 1999, 2000 and probably 2007, as well (MARAN-2008).

Figure 22 Daily dosages of antibiotics (calculated from the sold/delivered kg of active ingredient) per average animal per year in the various countries (MARAN-2007).

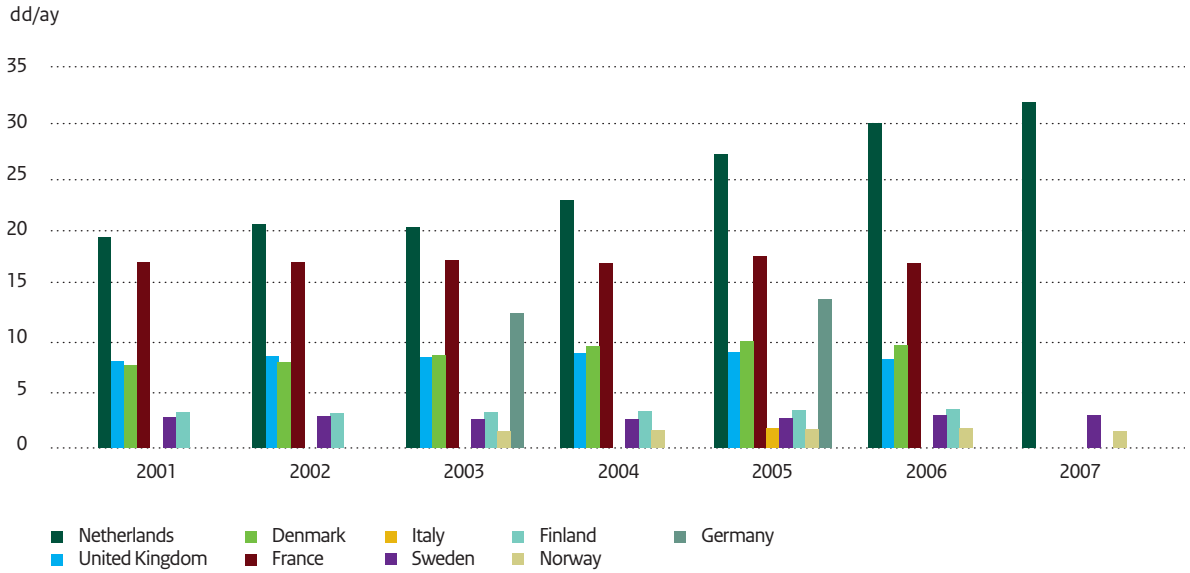
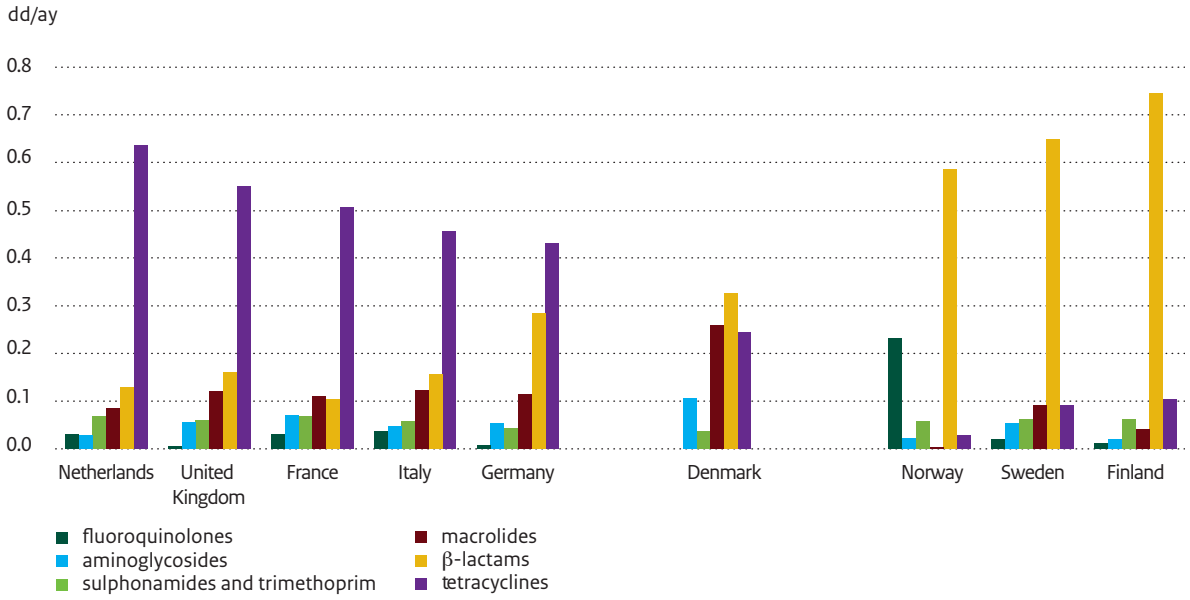


Figure 23 Percentages (%) of the total antibiotic use (expressed as calculated daily dosages) of the six main groups of antimicrobial preparations in each country in 2005 (MARAN-2007).



3.2 Resistance in food animals

Introduction

The increase in antibiotic resistance is a growing public health concern. The extent to which antibiotics are used for veterinary purposes on food animals can contribute to public and animal health risks. It is an important determinant for the development of antibiotic resistance within the treated animal populations. This is also recognized by the European Commission: the member states are required to monitor antimicrobial resistance in relation to public health. This chapter contains information about the monitoring results in animal populations in Europe, with special attention to the Netherlands. Furthermore, additional information is provided on fluoroquinolone resistance in *Campylobacter*, MRSA and the presence of ESBLs in *E. coli* and *Salmonella* isolated from animals.

Antimicrobial resistance standards and guidelines

The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is a standards-developing organization in the USA that publishes standards and guidelines for the antimicrobial susceptibility testing of bacteria. Although it has been used as an important guideline in many European countries, a number of countries had their own national antimicrobial breakpoint committees (the Netherlands, Germany, France, Norway, Sweden and the UK). In Europe, these committees had developed their national guidelines for antimicrobial susceptibility testing, each with their own defined antimicrobial Minimal Inhibitory Concentration (MIC) breakpoints for categorizing bacteria (and fungi) into susceptible, resistant and intermediate.

As a response, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) has initiated the development of collective European guidelines. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) was established as a coordinating organization to harmonise breakpoints for antimicrobial agents in Europe, and to act as the breakpoint committee for the European Medicines Agency (EMEA) during the registration of new antimicrobial agents.

Epidemiological cut-off values

In addition to clinical breakpoints for the guidance of therapy, EUCAST has defined separate breakpoints for the detection of bacteria with resistance mechanisms and the monitoring of resistance development (epidemiological cut-off values). In order to do this, it has established tables of wild type MIC distributions. Epidemiological cut-off values as defined by EUCAST enable the distinction of wild type organisms from non wild type microorganisms. A microorganism is defined as wild type (WT) (Kahlmeter et al., 2003) for a species by the absence of acquired and mutational resistance mechanisms to the drug in question. Hence, non-wild type organisms are defined as microorganisms with acquired or mutational resistance (Kahlmeter et al., 2003). Epidemiological cut-off values are important, as the application of only clinical breakpoints can mask important shifts in MICs towards a less susceptible population. When comparable methodologies are used, the MIC distribution of any given drug for the wild type population of any given microbial species is the same worldwide. The proportion of organisms no longer belonging to the wild type varies considerably and is – for many organism-antimicrobial combinations – increasing all over the world. The fact that the MIC distribution for a wild type microorganism is the same irrespective of when and where in the world it was collected and irrespective of whether the strain is of human or veterinary origin is fundamental for setting epidemiological cut-off values. EUCAST breakpoints tables and tables of wild type MIC distributions are freely available on its website (www.eucast.org).

Trends in antibiotic resistance in Dutch and European animal populations in general

In contrast to human patients, in intensively reared livestock, animals are usually not treated individually. The presence of high numbers of animals enhances the spread of infectious agents through a group or flock of animals. Therefore, when antibacterial therapy is applied in intensively reared animals, usually all animals in a group or flock are treated. This is done by medication in feed or water, exposing the intestinal flora to antibiotics, representing a huge number of bacteria. This enhances the chance that resistant microorganism are selected for. The high density of animals in intensive animal husbandry will also favour the dissemination of resistance genes among bacteria.

Although nowadays the use of growth promoters is prohibited by the EU, the addition of antibiotics to the feed of food animals has been widely used in the past. Table 5 shows some of the antimicrobial feed additives that have been used until 2006, when the European Union completely banned the use of antibiotics as growth promoters. Antimicrobial feed additives used as growth promoters, but also for therapeutic use, expose a huge amount of bacteria in the intestinal tract to antibiotics. Thus, selection pressure is not limited to animal pathogens, but is also reflected by the resistance levels in the normal commensal flora. Resistance levels in so called indicator microorganism such as *E. coli* and Enterococcus species are used to monitor the prevalence of resistance determinants in Gram-negative and Gram-positive bacteria respectively.

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (MARAN)

In the Netherlands, the results of the annual surveillance for antimicrobial resistance in a number of microorganisms (food pathogens, animal pathogens and indicator organisms) from various sources (pigs, poultry, cattle and food animal products) are published in an annual report, the Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (MARAN).

As part of MARAN, susceptibility profiles of commensal indicator organisms are monitored. The level of antimicrobial resistance in bacteria isolated from intestinal tract samples directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. For this purpose, *E. coli* and *Enterococcus* species (*E. faecium* and *E. faecalis*) are included as indicator organisms for the Gram-negative and the Gram-positive flora respectively. Isolation of bacteria from the intestine of randomly picked animals at slaughter aims to detect the development of decreased susceptibility at the bacterial population level in food animals.

From 2005 onwards, resistance in isolates from both dairy

Table 5 Antimicrobial feed additives approved for use in animals until 2006.

Antimicrobial drug	Antimicrobial class	Cross resistance to antimicrobials for therapeutic use	Banned in
avilamycin	orthosomycins	everninomycin	2005
avoparcine	glycopeptides	vancomycine, teicoplanine	1997
bacitracin	polypeptides	bacitracin	1999
flavophospholipol	glycolipids	-	2005
carbadox, olanquindoc	quinoxalines	-	
monensin, salinomycin	ionophores	-	2005
tetracycline?	tetracyclines	tetracyclines	1969
tylosin, spiramycine	macrolides	various macrolides (erythromycin, azithromycin, etcetera), lincosamides, streptogramins	1999
virginamycin	streptogramins	quinopristin/dalfopristin	1999

cattle and veal calves have been included in the monitoring, using samples that were taken at farms to determine the prevalence of *Salmonella*, *E. coli* O157 and *Campylobacter*.

Figures 24 to 26 illustrate trends over the years in levels of resistance in *E. coli*, *E. faecium* and *E. faecalis* strains of slaughter pigs, broilers, dairy cows and veal calves (MARAN-2008).

Resistance rates in *E. coli* continue to increase in slaughter pigs, broiler chickens and dairy cows. In broiler chickens, resistance in *E. coli* against the quinolones is disturbingly high and moreover still increasing. In 2008, more than 60% of all *E. coli* isolates were resistant against nalidixic acid and ciprofloxacin compared to almost 50% in 2006/2007. Also in veal calves resistance is high, but for most antibiotics that were tested, rates seem to either stabilize or show a moderate decrease. In dairy cattle, resistance in *E. coli* has been traditionally low, but is increasing alarmingly fast.
Multidrug resistance is increasing in all animal species tested with highest levels in veal calves and broilers. Another matter of concern is the emergence of extended-spectrum beta-lactamases (ESBL). ESBLs are detected in all *E. coli* of food-producing animals at low levels. The increase observed since 2003 in isolates from broiler chickens is alarming. In 2008, approximately 15% of the randomly isolated *E. coli* from chickens and chicken meat products were resistant against cefotaxime and ceftazidime, indicative of the frequent presence of ESBLs. In a recent prevalence study on 26 broiler farms it was determined that 100% of investigated farms were positive for ESBL-producing *E. coli* and that on 85% of these farms ≥80% (95% CI 71-99%) of the animals carried ESBL-producers in their faeces (MARAN-2008).

For both *E. faecalis* and *E. faecium,* high resistance levels were observed for tetracycline, erythromycin and streptomycin. Additionally, in *E. faecium* resistance rates are high for quinu/dalfopristin (73.8% vs. 1.9% in *E. faecalis*) and salinomycin (40% vs. 10.3% in E. faecalis). Ampicillin resistance was only observed in E. faecium. No resistance was observed against linezolid and florfenicol. Compared to previous years, the number of high level ciprofloxacin-resistant *E. faecalis* and *E. faecium* isolates (MIC ≥16 mg/l) in 2008 has increased in all considered farm animal species. Vancomycine resistance was observed in *E. faecium* strains isolated from all animal species included in this survey, although at a very low level.

It should be noted that the sampling strategy implies that this method is inherently insensitive for detecting resistance as only one randomly selected isolate is tested from a single sample taken from one animal per epidemiological unit (herd or flock). The total sample of selected isolates is intended to represent the *E. coli*, or *Enterococcus* species population of each animal species of the entire Netherlands. 1% resistance in e.g. *E. coli* indicates that in all animals 1% of the *E. coli* bacteria is resistant. Because each animal harbours about 106 cfu/g faeces *E. coli* in its gut, 1% would be approximately 104cfu/g faeces. This means that the absence of resistance in these datasets does not exclude the possibility that resistance is present in small numbers in each animal.

3.2.1 *Campylobacter*

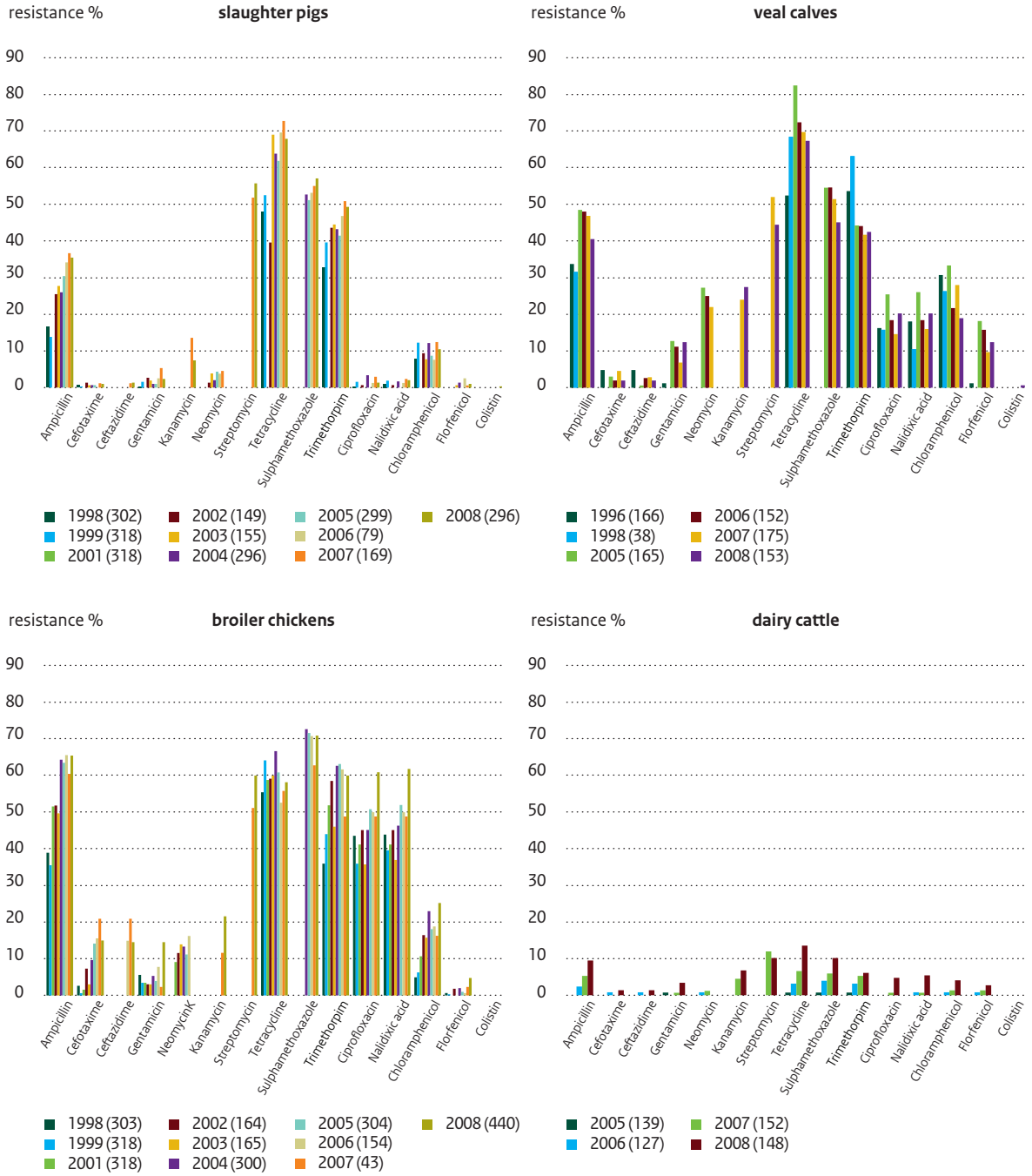
Introduction

Campylobacter has a broad animal reservoir and infection can result via numerous ways. However, poultry is still regarded as the main source for campylobacteriosis in human patients. Other routes of infection are via contaminated food, water, milk or the environment. Investigators from numerous countries have provided evidence suggesting that fluoroquinolone use in veterinary medicine, especially poultry, largely explains the increasing fluoroquinolone resistance among human *Campylobacter* isolates (Nachamkin et al., 2008)

Situation in the Netherlands

The fluoroquinole drug enrofloxacin has been registered

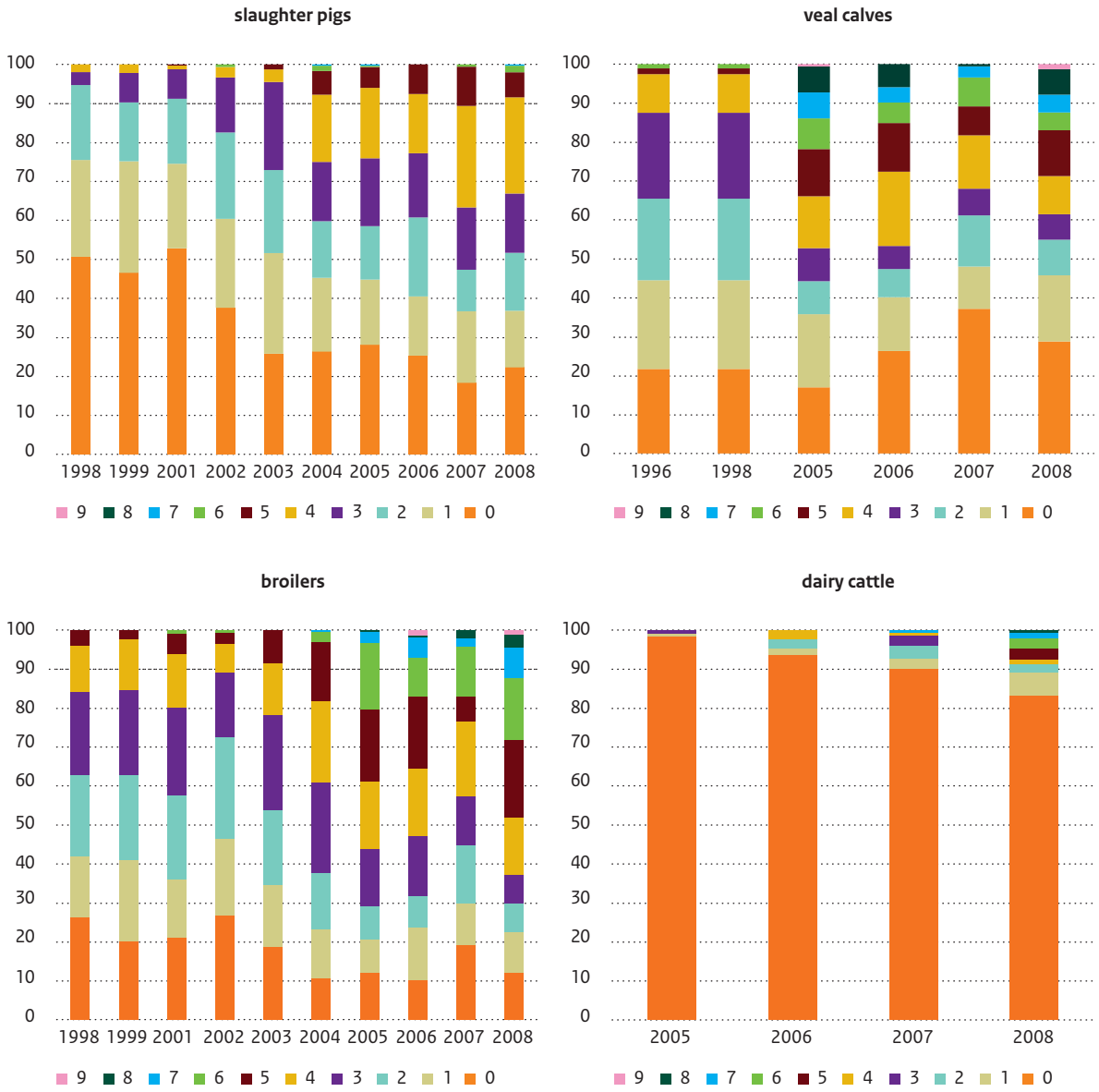
Figure 24 Trends in resistance (in %) of *E. coli* isolated from slaughter pigs and broilers in the Netherlands from 1998 – 2008 (MARAN-2008).



for the treatment of Gram-negative bacterial infections in poultry since 1987 in the Netherlands. In 1989, shortly after the introduction of ciprofloxacin for human use, resistance was observed in a substantial part of the *Campylobacter* isolates. 14% of the *C. jejuni* isolates from poultry and 11% of human isolates were resistant against ciprofloxacin (Endtz et al., 1991). In 1993, resistance levels in poultry had increased to 29% (Jacobs-Reitsma et al., 1994).
At present, *Campylobacter* resistance against the fluoroquinolones continues to increase in isolates from animals and from humans. In 2008, approximately 50% of the *Campylobacter* isolates from humans were resistant against ciprofloxacin, compared to 35% in the period 2002-2005. In broiler chickens more than 60% of the isolates were ciprofloxacin-resistant compared to 35-45% in 2002-2005 (Figure 27).

There is a clear distinction in resistance levels with regard

Figure 25 Trends in percentages of *E. coli* strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes in broiler chickens, slaughter pigs and veal calves in the Netherlands from 1996-2008 (MARAN-2008).



to the different food animal species, as shown in Figure 28. Resistance levels in *C. jejuni* and *C. coli* from Dutch food animals are highest in poultry and veal calves, while fluoroquinolone resistance in *Campylobacter* strains isolated from dairy cattle and pigs are relatively low.

In general, *C. coli* showed much more resistance and at higher levels than *C. jejuni*.

Situation internationally

In Europe, a steady increase in fluoroquinolone resistance in *Campylobacter* has been observed in many countries (De Jong et al., 2009).

In the European Union, Member States submit information on a yearly basis on the occurrence of zoonoses, zoonotic agents and foodborne outbreaks to the European Commission and the European Food Safety Authority (EFSA) in accordance with Directive 2003/99/EC. EFSA is responsible for examining the data on zoonoses, antimicrobial resistance and foodborne outbreaks. Results from Member States in 2008 are shown in Table 6 regarding antimicrobial resistance of *Campylobacter jejuni* and *C. coli* against fluoroquinolones as reported in the Community Summary Report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in the European Union in 2008: EFSA-Q-2009-00695. A wide

Figure 26 Trends in resistance percentages of E. *faecium* and E. *faecalis* isolated from slaughter pigs, broilers and veal calves in The Netherlands from 1996-2008 (MARAN-2008).

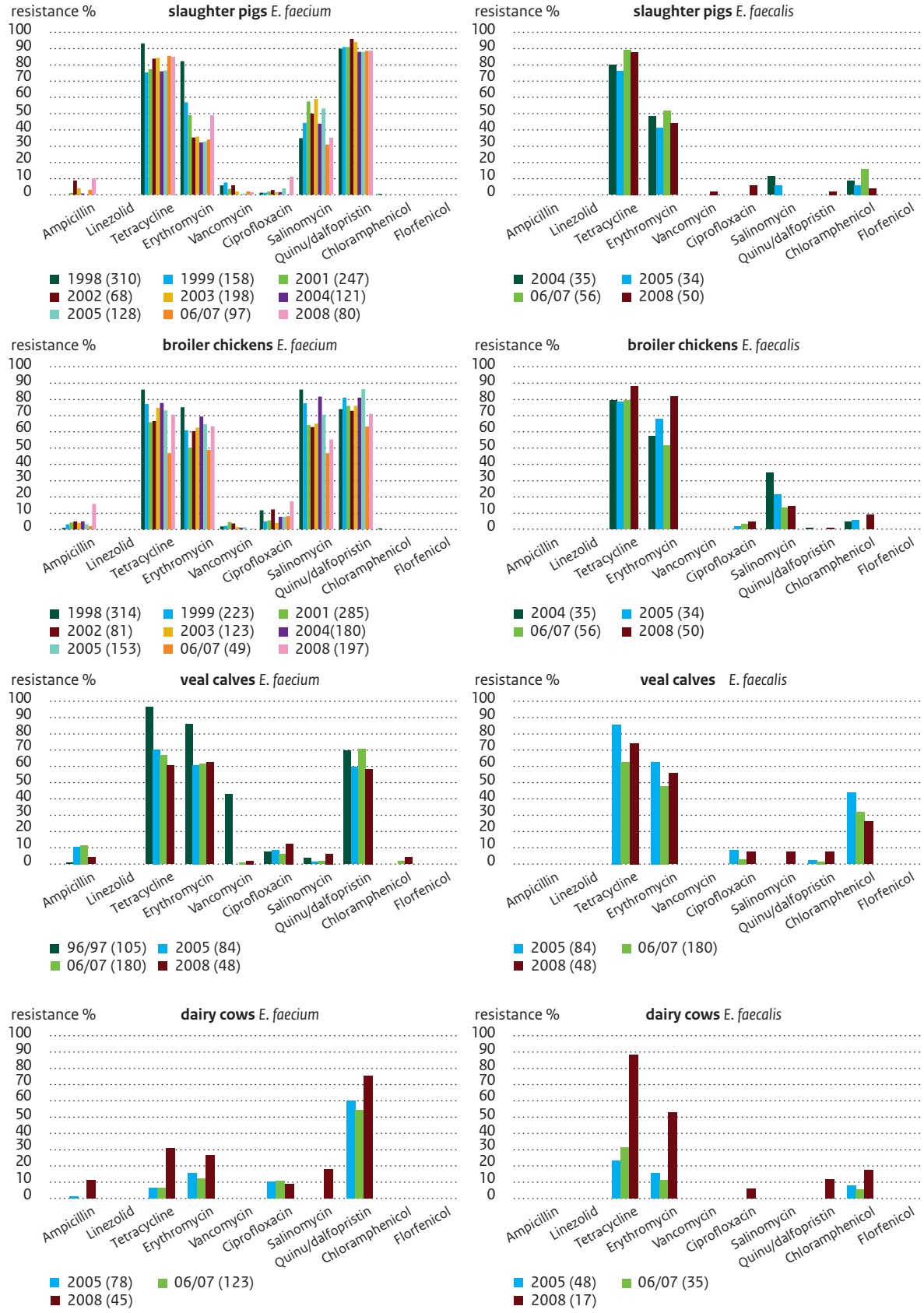


Figure 27 Trends in ciprofloxacin resistance in *Campylobacter jejuni* from broiler chickens in the Netherlands from 2000-2008 (Source: MARAN 2002-2008).

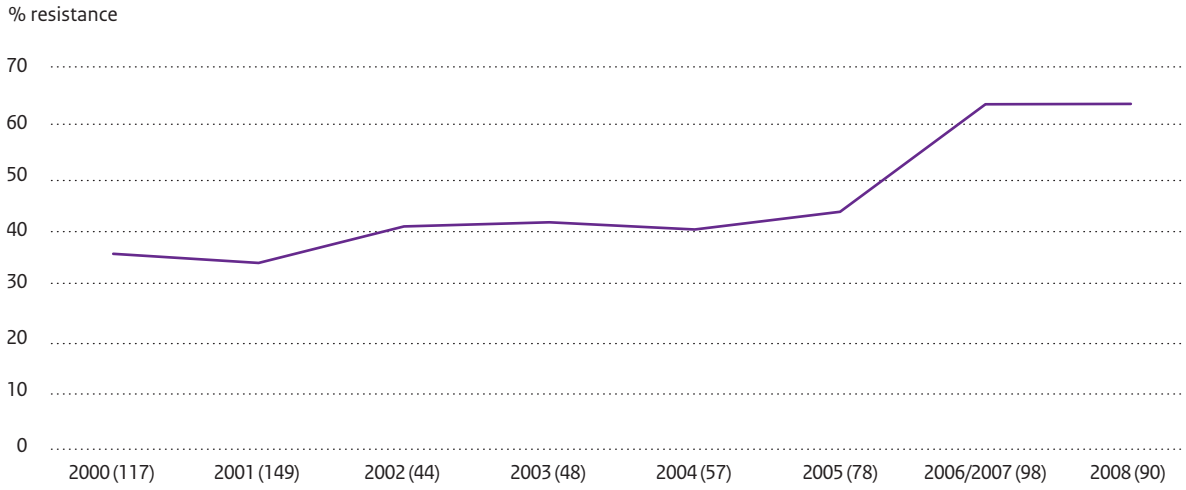
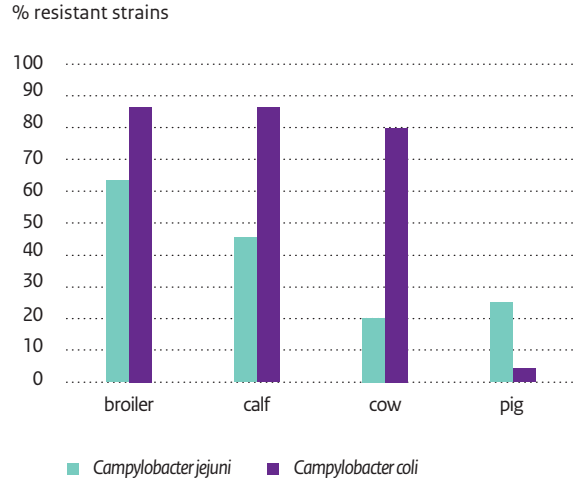


Figure 28 Ciprofloxacin resistance of *Campylobacter jejuni* and *C. coli* isolated from faecal samples of broilers, veal calves, dairy cows and pigs in 2007 (Based on data published in MARAN-2008).



range of resistance rates against fluoroquinoles are observed among *Campylobacter* strains isolated from animals in different countries.

In the USA and Canada, prior to 1992, fluoroquinolone resistance was rarely observed, but several reports have indicated that approximately 19-47% of *Campylobacter* strains from humans were resistant to ciprofloxacin (1997-2006, see Figure 29). Fluoroquinolone-resistant strains have also become prevalent in Africa and Asia. On both continents, fluoroquinolone resistance among clinical isolates was not detected prior to 1991. However, since 1993 the frequency of fluoroquinolone resistance has increased remarkably and the fluoroquinolone resistance rates have reached more than 80% in Thailand and Hong Kong. Although fluoroquinolone resistance was also observed in Australia and New Zealand the rate of fluoroquinolone-resistant *Campylobacter* isolates in this region is significantly lower than in other regions (Luangtongkum et al., 2009).

3.2.2 MRSA

Staphylococci form part of the normal flora in animals and *Staphylococcus aureus* is known to be able to cause a wide variety of pyogenic infections in a wide variety of animals. However, methicillin-resistant *Staphylococcus aureus* (MRSA) strains were considered typical human pathogens which were only sporadically detected in animals. Occasional reports described its isolation from intramammary infections in dairy cows, small companion animals or horses.

However since 2003, livestock-associated (LA-) MRSA has emerged in the human population. In the Netherlands, recent studies have shown that LA-MRSA is now widely present among livestock animals, especially in swine (Broens et al., 2008; De Neeling et al., 2007; Van Duijkeren et al., 2008) and cattle (Graveland et al, 2008; Olde Riekerink et al., 2009; Wagenaar and Van de Giessen, 2009). It is suggested that LA-MRSA originally were methicillin-susceptible commensal strains in pigs, whose spread was facilitated by the abundant use of antibiotics in pig and cattle farming (Voss et al., 2005). Indeed, a relation with the use of antibiotics in livestock farming has been implied in other reports (Van Duijkeren et al., 2007; Wagenaar and Van de Giessen, 2009).

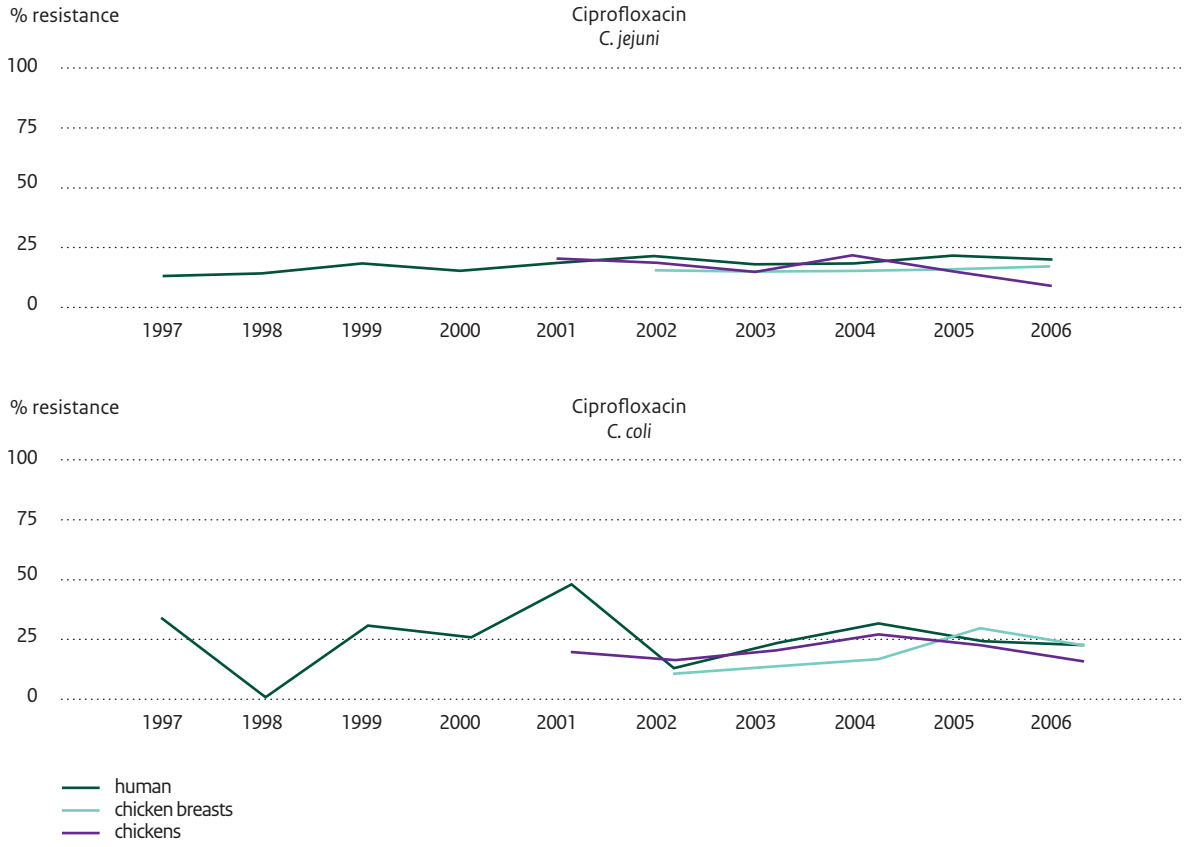
MRSA prevalence in the Netherlands

In the Netherlands, the majority of strains isolated from food animal species and food samples were identified as

Table 6 Antimicrobial resistance among Campylobacter isolated from animals (pigs and poultry) in different countries.

Country (year)	% resistant isolates (N)				Banned in
	Poultry			Pigs	
	C. jejuni	*C. coli*	Campylobacter spp	*C. coli*	
Czech Republic (2008)	56.4 (133)	-	-	-	(EFSA, 2009c)
Denmark (2008)	16.7 (150)	50.0 (6)	-	7.1 (98)	(Jensen and Hammerum, 2009)
France (2008)	30.7 (88)	62.4 (85)	-	-	(EFSA, 2009c)
Germany (2008)	-	-	56.9 (202)	-	(EFSA, 2009c)
Hungary (2008)	100 (110)	100 (110)		100 (41)	(EFSA, 2009c)
Italy (2008)	80 (55)	88.9 (54)	-	-	(EFSA, 2009c)
Latvia (2008)	100 (57)	100 (110)	-	-	(EFSA, 2009c)
Poland (2008)	81.9 (105)	81.5 (65)	-	-	(EFSA, 2009c)
Portugal (2008)	95.5 (134)	96.3 (214)	-	-	(EFSA, 2009c)
Romania (2008)	77.6 (134)	83.1 (83)	-	-	(EFSA, 2009c)
Slovenia (2008)	70.1 (97)	60.9 (69)	-	-	(EFSA, 2009c)
Spain (2008)	37.6 (125)	40.2 (214)	-	92.5 (93)	(EFSA, 2009c)
Slovenia (2008)	70.1 (97)	70.0 (60)	-	-	(EFSA, 2009c)
Switzerland (2008)	18.3 (115)	18 (50)	-	-	(EFSA, 2009c)
UK (2008)	18.5 (130)	25.0 (40)	-	17.1 (287)	(EFSA, 2009c)
USA (2007)	32.1 (78)	14.6 (28)	-	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18125

Figure 29 Antimicrobial resistance among *Campylobacter* isolates from humans, retail meats and chickens, 1997-2006. From: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm182987.htm



sequence type (ST)398, belonging to the clonal complex (CC)398. This clone is (internationally) related to farm animals. However, also a limited number of sequence types have been identified that are typically related to human healthcare MRSA. These were isolated from pig, veal and poultry farms.

Pigs

After the discovery of the high prevalence of MRSA in pig and later also in veal calf farming as well as in persons in contact with these animals, like veterinarians and farmers, an extensive research program was conducted in the Netherlands to investigate the risk factors for MRSA in Dutch livestock farming. In this research program intensive prevalence studies were performed in different animal species. Among pig farms, 68.3% of 202 farms tested positive for MRSA. A risk factor analysis conducted on the 171 sow farms indicated that the prevalence of MRSA was higher on large farms (>500 sows) compared to small farms (<250 sows). Additionally, the prevalence of MRSA increased over time (from 30% in early 2007 to 75% in late 2008). Other factors, such as the presence of finishing pigs, the purchase of gilts, the farm hygiene score or the antibiotic use were not statistically significant, but were all associated with farm size. Within the pig production pyramid, there is evidence of a clear association between the MRSA status of the supplier and the MRSA status of the purchaser. Moreover, the findings indicated that MRSA-negative animals can become MRSA-positive within a few hours of being placed in a contaminated environment, such as a contaminated transport lorry or lairage (Wagenaar and Van de Giessen, 2009).

Veal calves

Studies conducted in veal farms indicated that MRSA was present in 88% of the 102 examined farms with 27.5% of the veal calves found positive. Significant positive associations were found between the presence of MRSA in calves and the age of the animals, the number of calves per section, the presence of other farm animals, the use of pest control for rats and mice and the use of antibiotics. However, the presence of MRSA in calves was negatively associated with the number of animal buildings on a farm, cleaning and disinfection of buildings (Wagenaar and Van de Giessen, 2009).

Poultry

Out of the 40 broiler flocks that were examined in poultry slaughterhouses, 35% tested positive for MRSA. Of the individual animals that were tested 6.9% were found to be MRSA throat carriers (Wagenaar and Van de Giessen, 2009).

Dairy cows

In a prevalence study conducted in the Netherlands in 2007-2008, MRSA was found in 4 of 200 randomly selected dairy herds in animals with intramammary infections (IMI) (as determined on basis of elevated somatic cell counts), indicating a herd prevalence of 2%. Prevalence in all sampled cows (n= 2873) was 0.2% and 0.04% for all cows in the sampled herds. A significant association was found between MRSA IMI in dairy cattle and the presence of commercially kept swine on the same farm (Odds Ratio 6.3, CI 3.1-12.8). (Olde Riekerink et al., 2009).
Also in a yearly performed survey on the resistance profiles of mastitis pathogens, MRSA is incidentally observed in *S. aureus* isolated from milk samples obtained from cows with intramammary infections (MARAN-2007; MARAN-2008). Genotyping results of isolates from dairy cows are not available.

Pet animals and horses

Up to 2006, MRSA was rare in pets and horses but is now increasing (Van Duijkeren et al., 2010). At the Veterinary Microbiological Diagnostic Centre in the Netherlands, the percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates found in equine clinical samples increased from 0% in 2002 to 37% in 2008. MRSA of spa-type t064, belonging to Multilocus sequence type (MLST) ST8 and spa-types t011 and t2123, both belonging to the livestock-associated MLST ST398, predominated. Data from several outbreaks of post-surgical MRSA infections in horses indicated that nosocomial transmission occurs in equine clinics and suggests that personnel play a role in the transmission (Van Duijkeren et al., 2010).

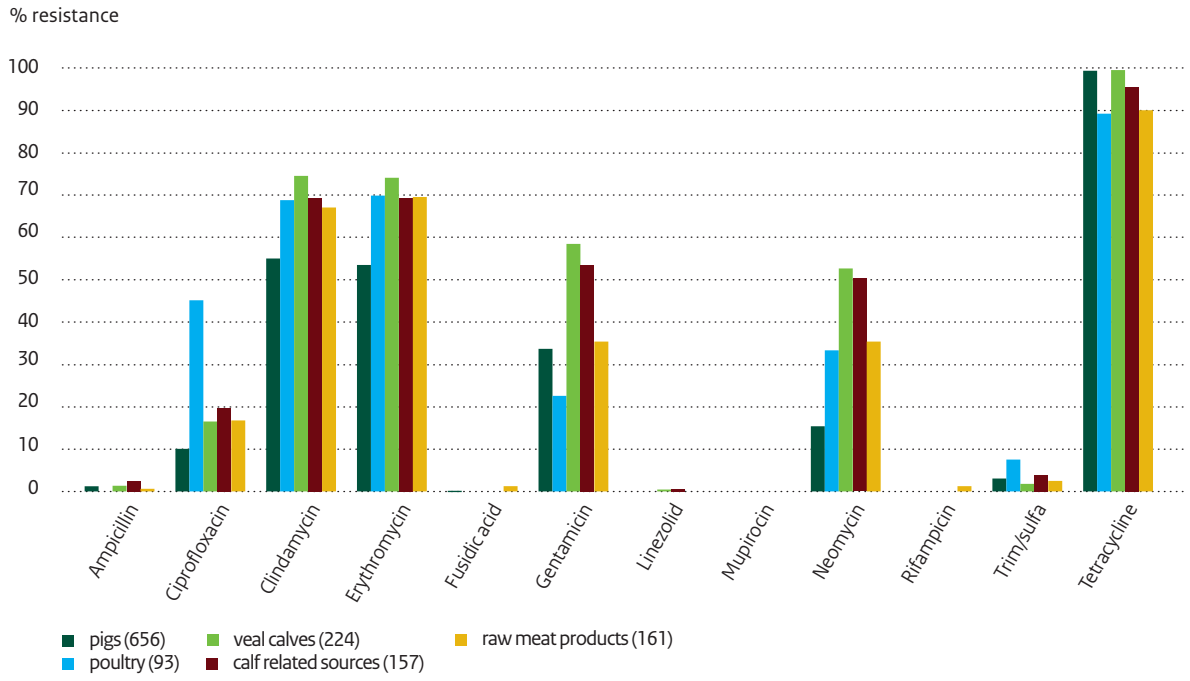
Resistance profiles in animal MRSA in the Netherlands

As part of the Dutch MRSA research program intensive MRSA prevalence studies were performed in different animal species and all isolates were sent to to the Central Veterinary Institute (CVI-Lelystad) for susceptibility testing. The purpose was to determine if next to the *mec*A-gene cluster additional resistance genes had been acquired as a result of different antibiotic use practices in food animals. This is considered important because of the zoonotic potential of animal related MRSA.
Resistance profiles were determined of 1290 MRSA isolated from food animals (Table 7 and Figure 30). Most isolates (97%) were tetracycline-resistant. This was expected as tetracycline resistance is a characteristic for MRSA ST398, based on the presence of a tetM gene (Witte et al., 2007). Therefore it was remarkable that also tetracycline susceptible isolates were observed, about 10% of the isolates from poultry and meat products. For the tetracycline susceptible isolates from meat products, it was shown that these belonged to MLST types other than type 398 (De Boer et al., 2009). No information is available regarding the MLST types of the tetracycline susceptible poultry isolates.

Table 7 MIC distributions (mg/L) as determined for 1290 animal related MRSA isolates in the Netherlands, concerning 12 antibiotics (Wagenaar and Van de Giessen, 2009).

	Total	MIC (mg/L) distribution (%)													
Breakpoint	**N = 1290**	**0,06**	**0,13**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
R > 16	Amikacin				0,2	2,1	26,7	35,7	24,4	9,7	1,2				1,2%
R > 1	Ciprofloxacin				77,4	6,9	6,9	0,6	1,4	3,7	1,8	1,1	0,2		15,7%
R > 4	Clindamycin			34,2	2,6	0,6		0,1	0,9	1,2	0,6	59,8			62,6%
R > 8	Erythromycin			9,5	27,1	1,2						62,1			62,2%
R > 8	Fusidic acid			64,4	33,1	1,9	0,1	0,3	0,2						0,2%
R > 1	Gentamicin				49,6	10,6	2,1	0,9	0,5	1,1	6,7	18,1	10,3		39,8%
R > 4	Linezolid					1,4	45,5	52,9	0,2						0,2%
R > 4	Mupirocin				97,4	1,9	0,7								0,0%
R > 8	Neomycin				29,5	25,1	11,9	3,5	10,7	9,9	6,4	2,1	0,8		29,9%
R > 4	Rifampicin			98,7	0,9	0,2									0,2%
R > 4/76	Trim/sulfa		34,7	5	21,9	27,5	7,8	2,2	0,2	0,1	0,7				3,2%
R > 16	Tetracycline				2	0,7		0,2		0,3	2,2	20,8	73,8		97,1%

Figure 30 Resistance percentages regarding 12 antibiotics with respect to animal related MRSA isolated from different sample sources in the Netherlands (Wagenaar and Van de Giessen, 2009).



Additionally, high levels of resistance levels were observed for the macrolides as 62.2% of the MRSA isolates had an MIC exceeding 32 mg/l for erythromycin, which classified them as resistant.

Also high levels of resistance were observed against clindamycin (62.6%). Compared to previous findings in pig isolates (De Neeling et al., 2007) these data reflect an increase in resistance against lincosamides. This is an important trend which affects the preference of antibiotics for therapeutic treatment in human patients. In hospital settings, clindamycin has been advised as empirical treatment for animal related MRSA infections. This advice was already questioned by Renders et al., which is supported by these data (Renders et al., 2007).

Resistance against the aminoglycosides (gentamicin and neomycin) showed considerable variation (from 15 to 57%) among the various sample sources. Highest levels of resistance against gentamicin and neomycin were found in veal calves. This has also been described in studies in other countries.

Levels for ciprofloxacin resistance varied (10-45%), showing highest levels in MRSA from poultry. This can be

explained by the extent of fluoroquinolone use in this animal production sector. Resistance against ciprofloxacin occurs spontaneously owing to point mutations in the chromosome and subsequently, resistant isolates will be selected for when exposed to fluoroquinolones. Antibiotic treatment with fluoroquinolones is quite common in both veal calve and poultry sector.
Moreover, levels of resistance against ciprofloxacin show an increase over the years. In a previous study by the RIVM, all MRSA isolates were still susceptible for this antibiotic (De Neeling et al., 2007).
Resistance against the trimethoprim/sulphamethoxazole combination was generally low. This is remarkable, as these antibiotics are widely used for the treatment of food animals. A possible explanation is that clinical resistance requires the presence of two genes (one coding for resistance against trimethoprim together with a gene for resistance against sulphonamides), both of which have rarely been described in *Staphylococcus aureus* (Kadlec and Schwarz, 2009).
Animal MRSA isolates showed either no or sporadic resistance against vancomycine, mupirocine, fusidic acid and rifampicine, antibiotics that are considered important drugs for the treatment of MRSA in human patients.

Multidrug resistance was wide spread in animal MRSA. Multiresistance was generally found against beta-lactam antibiotics, macrolides, lincosamides, aminoglycosides (neomycin and gentamicin) and fluoroquinolones.

MRSA resistance in other countries

Pigs

To assess the occurrence and the diversity of MRSA in pig primary production, a European Union-wide preliminary survey was carried out in parallel with a baseline survey on *Salmonella* spp. in holdings with breeding pigs to determine the prevalence of holdings positive for MRSA and MRSA ST398 in 2008. In this study, 17 Member States detected MRSA in their breeding or production holdings whereas 7 Member States did not detect any MRSA in the surveyed holdings. MRSA was more often detected in production holdings than in breeding holdings. The European Union prevalence of MRSA-positive holdings with breeding pigs, as estimated based on the results from the 24 participating Member States, was 22.8%. The European Union prevalence of MRSA and of MRSA ST398-positive production holdings was 26.9% and 25.5%, respectively. MRSA prevalence varied widely among the Member States, from 0% to 51.2%. Highest prevalence was detected in Italy, Germany, Spain and Belgium. MRSA ST398 was the predominant MRSA lineage identified in the European Union, counting for 92.5% of the MRSA isolates (EFSA, 2009b).

Also outside Europe countries have confirmed the finding of high prevalence of MRSA ST398 in pig farming, e.g. Canada, Singapore and USA (Catry et al., 2010; Vanderhaeghen et al., 2010). The reported prevalence varies between countries, ranging from 1-40% at pig level and up to 68% at farm level. This may be influenced by differences in set up of the studies and in isolation techniques. In general, LA-MRSA seems to be the predominant strain in pigs, but recent investigations in Asia have demonstrated that pig farms can also act as a reservoir for other sequence types of MRSA, e.g. ST9 in China and Malaysia (Catry et al., 2010).

Poultry

Other than in the Netherlands, in Belgium ST398 MRSA has been isolated in broilers in up to 14.3% of the broiler farms (Catry et al., 2010). Information on MRSA in poultry in other countries is currently lacking.

Veal calves

Other then in the Netherlands, information on prevalence of MRSA in veal calves is sparse.

Dairy cows

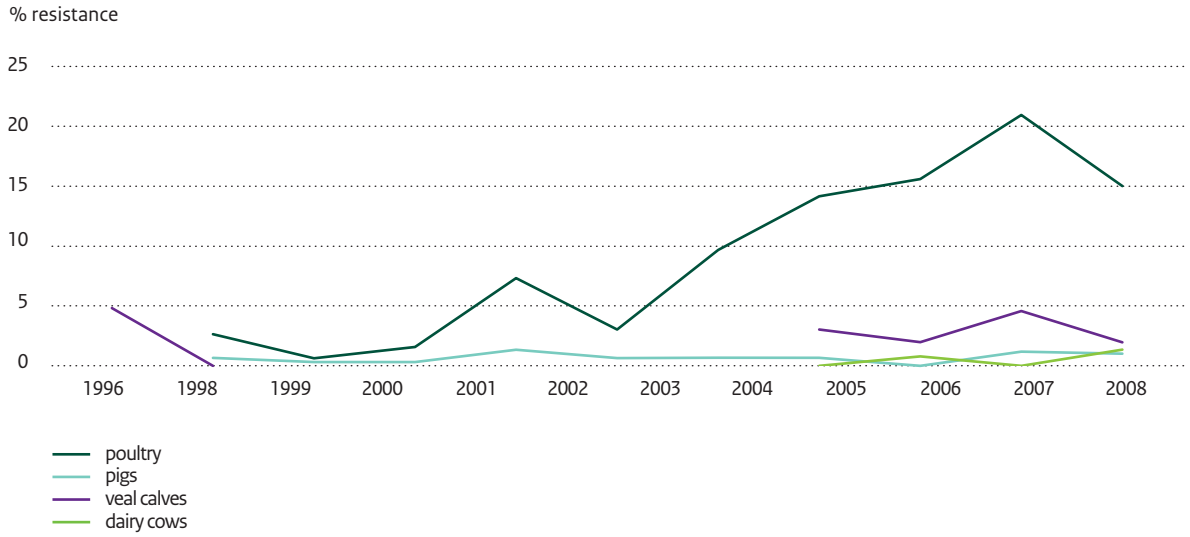
In general, like in the Netherlands, the prevalence of MRSA in bovine mastitis cases seems to be very low (Hendriksen et al., 2008; Vanderhaeghen et al., 2010). In a recent study in Belgium however, LA-MRSA was found in 10% of the farms with mastitis caused by *Staphylococcus aureus* with prevalence on positive farms of 3.9-7.4% of the animals (Vanderhaeghen et al., 2010).

Pet animals

Epidemiological evidence, including phenotypic and molecular typing data, suggests that MRSA isolates from dogs and cats are indistinguishable from human healthcare isolates, whereas strains of MRSA isolated from horses are different (Leonard and Markey, 2008).
Carriage in healthy dogs is still rare but it can be found in 2% of those with skin conditions. There are occasional outbreaks seen in some animal hospitals, including those treating horses, with the majority of these infections appearing to be caused by the same strains that are seen in humans (Hunter et al., 2010). Although infections and carriage in pets is low, the strains are often the same as those commonly found in humans and transmission from humans to pets and vice versa is likely (Strommenger et al., 2006)

In a Danish study, MRSA carriage was significantly ($P<0.02$) higher among the veterinary practitioners (3.9%) than among the participants not professionally exposed to animals (0.7%). Six of the nine MRSA strains isolated from veterinary practitioners belonged to clonal complexes (CC) previously associated with horses (CC8), small animals

Figure 31 Cefotaxime resistance in E. coli from different animal species in the Netherlands from 1996-2008 (based on MARAN 2002-2008).



(CC22), and pigs (CC398). Although four of the nine positive veterinarians carried the CC associated with pigs, exposure to small animals, cattle, or horses, but not to pigs, was found to be a significant risk factor. The results indicate that veterinarians are at risk of MRSA carriage (Moodley et al., 2008).

In Germany, limited data are available on the occurrence of MRSA in dogs and cats. However, oxacillin resistance levels among coagulase-positive staphylococci (*S. aureus* and *S. pseudintermedius*) was rarely observed (<2%) (Kresken, 2009).

3.2.3 ESBL-producing bacteria

Introduction

As for MRSA, beta-lactamase resistance in animal-derived bacteria has shown a clear increase during the past decade. Beta-lactam antibiotics constitute one of the most important classes of antimicrobial used in veterinary medicine with a wide assortment of beta-lactam drugs that are licensed for the treatment of animals.
Although beta-lactam antibiotics are among the oldest antimicrobials and have been used for decades, the development of the newer generations of cephalosporins with their broad (extended) spectrum against both Gram-positive and Gram-negative bacteria has probably been the driving force for the recent emergence of ESBLs. ESBLs have first been described a few years after the third generation of cephalosporins were licensed for therapeutic use in humans. Also in veterinary medicine, the increase in ESBLs shows parallels with the availability of third and fourth generation cephalosporins in the veterinary field.

ESBLs in the Netherlands

Escherichia coli

In the Netherlands, ESBLs in *E. coli* have been identified in all food-producing animals at low levels. However, in isolates from broiler chickens a strong increase has been observed in the last decade. In 2008, approximately 15% of the randomly isolated *E. coli* from chickens and chicken meat products were resistant against cefotaxime and ceftazidime, indicative of the frequent presence of ESBLs (MARAN-2008). In Figure 31, the level of resistance against cefotaxime is shown in *E. coli* isolated from different animal species.

Salmonella

In 2008, a variety of *Salmonella* serovars were found to be cefotaxime resistant. The majority of isolates consisted of the serovar *S.* Java (45 of the 65 isolates), which is the most predominant *Salmonella* serovar in poultry in the Netherlands. Other ESBL-suspected serovars were Agona, Infantis, Senftenberg, Typhimurium Ft90, Enteritidis PT4, Virchow, Kottbus, Cubana, Rissen and Heidelberg (MARAN-2008).
Up to now, poultry is the only reservoir for ESBL-producing *Salmonella's* in animals, which is associated with the transmission of the genetic determinant between *Salmonella* and ESBL-producing *E. coli* in poultry. Third generation cephalosporins are not used in broiler production, but the use of ceftiofur in combination with Marek vaccine or with in-ovo vaccination has been a common off-label use procedure in the poultry reproduction and breeding sectors. Possibly, this has contributed to selection and vertical transmission of ESBLs in the poultry production pyramid. However, co-resistance to non-beta-lactam antibiotics in ESBL-producing

Enterobacteriaceae is commonly described and co-selection through usage of other drugs may have played a role in the selection for ESBL-producing isolates (Dierikx et al., 2010).
The *S.* Java isolates from poultry are clonally related and totally adapted to poultry, rarely causing human infection. The clone is typically resistant against trimethoprim and streptomycin (low level), but has in addition acquired many other resistance determinants: besides ESBLs, also tetracycline resistance genes and chromosomal and plasmid mediated quinolone resistance has been reported (Hasman et al., 2005).

ESBLs in other countries
Since the start of the 21st century there has been an explosive increase in the prevalence ESBL-producing bacteria worldwide, particularly those that express the CTX-M beta-lactamases. Such strains are usually isolated from humans. However, some animal isolates also produce CTX-M enzymes (Hunter et al., 2010). Although a wide range of ESBLs has been detected in bacteria isolated from veterinary samples, reports of clinical infections due to ESBL-producing strains in animals have so far been very limited, with only low numbers of animals affected (Hunter et al., 2010).

E. coli carrying an ESBL were first detected in livestock in the UK in diarrhoeic calves on a Welsh dairy farm in 2004. The ESBL present was CTX-M-14. Examination of bovine veterinary diagnostic samples tested at the Veterinary Laboratories Agency in England and Wales between 2006 and 2007 showed that the predominant enzymes produced by veterinary isolates were CTX-M-14 and CTX-M-15, which are also the predominant ESBL-types isolated from human isolates (Hunter et al. 2010).

E. coli isolates producing the CTX-M-1 enzyme have been reported in food animals in Denmark, Spain and France. CTX-M-1 has also been identified in pet animals in Italy. The majority (76%) of the cefotaxime resistant *E. coli* isolates recovered from both healthy and diseased dogs and cats during 2001-2003 were CTX-M-1 positive (Carattoli, 2008).

In Europe, next to the Netherlands, CTX-M-2 producing *S.* Virchow have been reported in poultry in a number of other countries e.g. the UK, France and Belgium (Bertrand et al., 2006; Hopkins et al., 2006).

Narrow spectrum SHV-1-, TEM-1-, and OXA-type beta-lactamases have been frequently described in *E. coli* and *Salmonella* spp from animals and food of animal origin in Spain, Germany, the UK and the USA. TEM-1 was the most common variant among these isolates, but it was only the last few years that some ESBLs known to be relevant to human medicine have been described in isolates from animals (Carattoli, 2008).

Although the ability of bacteria to produce ESBLs has to be confirmed by molecular methods, their presence is indicated by resistance to third and fourth generation cephalosporins. An overview of published reports on such resistance in veterinary samples is shown in Table 8 (*E. coli*) and Table 9 (*Salmonella*). These tables include the 2008 results from European Union Member States with regard to antimicrobial resistance against third generation of cephalosporins in *Salmonella* and indicator *E. coli* as reported in (EFSA, 2009c). It has to be kept in mind that the antimicrobial susceptibility testing information reported was not representative of the whole of the EU. In general, the proportion of resistant isolates to third generation cephalosporins in the reporting MSs was very low. Resistance levels are highest in poultry, but are also present in other food animals.

USA
Among *Salmonella* (non-Typhi) isolates resistance against third generation of cephalosporins (ceftiofur) has been observed in poultry (broiler chickens and turkeys), cattle and swine. Highest prevalences are observed in chickens and cattle and in both animal species a clear increase is observed from 1996 on (Figure 32).
In 2006, 12.9% of the tested *Salmonella* species from chickens showed a MIC >2 mg/L against ceftiofur, 5% of turkeys, 18.8% of cattle and 2.2% of swine isolates. Most prevalent serovars isolated from chickens were *S.* Kentucky (58.2%), *S.* Typhimurium (18.1%), *S.* Heidelberg (14.7%) and *S.* I 4,[5],12:i:- (7.3%). In cattle the most prevalent serovars were *S.* Newport (31.5%), *S.* Reading (21.9%), *S.* Agona (9.6%), *S.* Dublin (8.2%) and *S.* Typhimurium (8.2%).
When ceftiofur resistance is regarded in different serovars, high percentages are found in *S.* Typhimurium isolates from both chickens and cattle, roughly 30% of the isolates was ceftiofur resistant, compared to 80% of *S.* Newport strains from cattle.

3.3 Resistance in foods of animal origin

Fluoroquinolone-resistant *Campylobacter*
Table 10 shows an overview of the percentage of *Campylobacter* isolates recovered from food samples with resistance against fluoroquinolones in different countries. In line with resistance among *Campylobacter* isolates from live animals, resistance rates in animal food samples differ greatly between countries, with reported resistance rates up to 100% in Latvia and Portugal (Table 10).
Although data concerning both live animals and food of animal origin are available from only a limited number of countries, in general resistance rates between both sources are similar (Table 6 and Table 10).

Table 8 Resistance against third generation cephalosporins in *E. coli* from food animals in different countries.

E. coli		% resistant isolates (N)				
Country (year)	**Antimicrobial tested (breakpoint used)**	**Pig**	**Cattle**	**Poultry**	**Turkey**	**Reference**
Netherlands (2008)	cefotaxime (>0,25 mg/l)	1.0 (296)	1.4 (148)	15 (440)	-	(MARAN-2008)
Denmark(2008)	cefotaxime (>0,25 mg/l)	0 (151)	0 (97)	0 (114)	-	(Jensen and Hammerum, 2009)
France (2008)	cefotaxime(>0,25 mg/l)	0 (137)	0 (118)	4.8 (146)	0.8 (246)	(EFSA, 2009c)
Germany (2008)	ceftazidime (>2 mg/l)	0 (37)	1.3 (76)	16.7 (6)	-	(EFSA, 2009c)
Spain(2008)	cefotaxime(>0,25 mg/l)	0.6 (168)	0 (168)	30.1 (113)	-	(EFSA, 2009c)
Sweden(2008)	cefotaxime (>0,25 mg/l)	0 (349)	-	-	-	(Bengtsson et al., 2009)
Switzerland (2008)	ceftiofur (>4 mg/l)	-	0 (80)	0 (149)	-	(EFSA, 2009c)
UK (2008)	cefotaxime (>0,25 mg/l)	0.9 (215)	9.0 (1942)	1.0 (96)	0 (29)	(EFSA, 2009c)
Canada (2008)	ceftiofur (>4mg/l)	0.7-1.1* (1575)	0 (176)	20 (170)	-	(Anonymus, 2008)
	ceftriaxone(>32 mg/l)	0 (1575)	0 (176)	0 (170)	-	(Anonymus, 2008)
USA (2008)	ceftiofur (>8 mg/l)	-	-	0 (986)	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18127
	ceftriaxone (>32 mg/l)	-	-	10.4 (986)	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18127

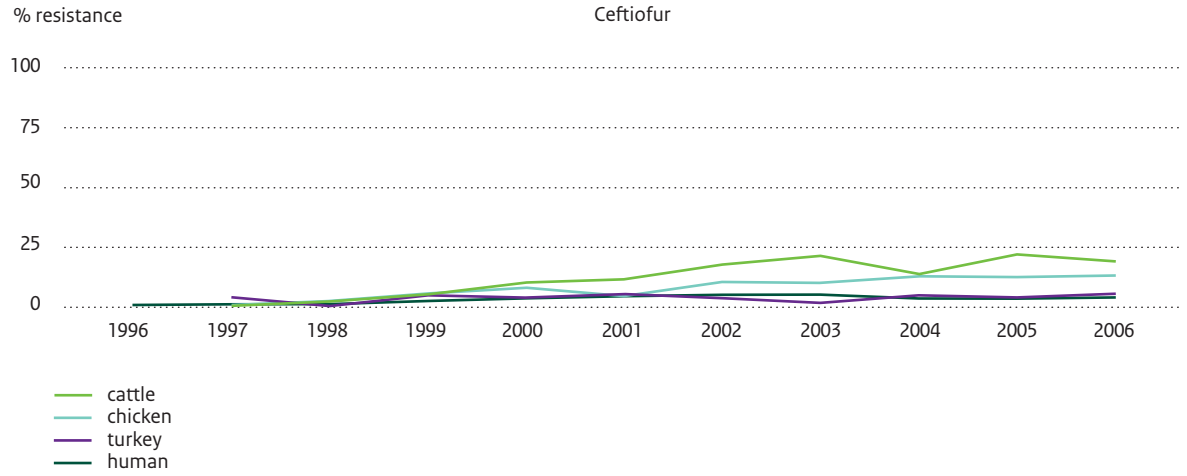
* depending on province or region

Table 9 Resistance against third generation cephalosporins in *Salmonella* from food animals in different countries.

Salmonella		% resistant isolates (N)					
Country (year)	**Cephalosporin tested (breakpoint used)**	**Pig**	**Cattle**	**Broilers**	**Laying hens**	**Turkey**	**Reference**
Netherlands(2008)	cefotaxime (>0,5 mg/l)	0 (313)	0 (47)	-	15.3 (137)	0 (28)	(EFSA, 2009c)
Belgium (2008)	cefotaxime (>0,5 mg/l)	0 (354)	2 (43)	-	0 (50)	6.7 (208)	(EFSA, 2009c)
Denmark(2008)	cefotaxime (>0,5 mg/l)	0.8 (497)	0 (18)	-	-	-	(EFSA, 2009c)
France (2008)	cefotaxime (>0,5 mg/l)	0.9 (111)	-	-	0 (27)	1.1 (186)	(EFSA, 2009c)
Germany(2008)	ceftazidime (>2 mg/l)	0.4 (518)	0 (334)	-	0.5 (364)	0 (37)	(EFSA, 2009c)
Italy (2008)	cefotaxime (>0,5 mg/l)	1 (106)	0 (16)	-	33.3 (24)	0 (23)	(EFSA, 2009c)
Poland (2008)	cefotaxime (>0,5 mg/l)	0 (115)	-	0 (977)	-	-	(EFSA, 2009c)
Spain(2008)	cefotaxime (>0,5 mg/l)	11 (61)	-	-	-	0 (17)	(EFSA, 2009c)
Sweden (2008)	cefotaxime (>0,5 mg/l)	0 (25)	0 (39)	0 (16)	-	-	(Bengtsson et al., 2009)
Switzerland(2008)	ceftiofur(>4 mg/l)	3 (30)	-	-	-	-	(EFSA, 2009c)
UK (2008)	cefotaxime (>0,5 mg/l)	0 (404)	0 (76)	-	0 (20)	0 (19)	(EFSA, 2009c)
Canada (2008)	ceftiofur (>4mg/l)	0-1.3* (367)	4.5 (133)	11.5 (234)	-	-	(Anonymus, 2008)
	ceftriaxone (>32 mg/l)	0 (367)	0 (133)	0 (234)	-	-	(Anonymus, 2008)
USA (2008)	ceftiofur(>8 mg/l)	4.5 (111)	16.3 (443)	8.7 (624)	-	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18127
	ceftriaxone (>32 mg/l)	0.9 (111)	1.4 (443)	0.3 (624)	-	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18127

* depending on province or region

Figure 32 Antimicrobial resistance among *Salmonella* (non-Typhi) isolates from humans and food animals by year, 1996-2006. From: website FDA.



(LA-) MRSA

The finding of MRSA in food animals, specifically a specific clone, multilocus sequence type 398 (ST398), which has been found in up to 11.9% of retail meat samples in several surveys from different parts of the world, has led to concerns about the risk of food as a reservoir for human infections (Kluytmans, 2010).

An overview of the current literature on MRSA in food is presented in Table 11. Although some studies specifically address ST398, the overview includes all MRSA found in samples from food animals. Additionally, we like to refer to an extensive review on the assessment of the public health significance of MRSA in food as released by the European Food Safety Authority (EFSA, 2009a).

ESBL-producing bacteria

Although the ability of bacteria to produce ESBLs has to be confirmed by molecular methods, their presence is indicated by resistance to third and fourth generation cephalosporins. An overview of published reports on such resistance in food samples is shown in Table 12 (*E. coli*) and Table 13 (*Salmonella*). These tables include the 2008 results from European Union Member States with regard to antimicrobial resistance against third generation of cephalosporins in *Salmonella* and indicator *E. coli* as reported in the Community Summary Report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in the European Union in 2008: EFSA-Q-2009-00695.

It has to kept in mind that the antimicrobial susceptibility testing information reported was not representative of the whole of the EU. In general, the proportion of resistant isolates to third generation cephalosporins in the reporting Member States was very low. Similar to the situation in live animals, resistance levels are highest in poultry meat, although also present in food samples from other food animals.

In the Netherlands, ESBL-producing bacteria were detected in 45.9% of 249 meat samples. ESBL was most frequently recovered from chicken (88.0%), but was also recovered from beef (18.7%) and pork (18.9%), (Overdevest and Kluytmans, 2010).

3.4 Other reservoirs of resistance

3.4.1 The environment

The environment is considered to be the natural reservoir of resistance genes. Soil bacteria, especially bacteria belonging to the family of Actinomycetes, are known to produce antibiotic resistance proteins to protect themselves from antibiotics they produce themselves (Benveniste and Davies, 1973; Cundliffe, 1989; D'Costa et al., 2006; Hopwood, 2007; Tahlan et al., 2007). Possibly, antibiotics produced by these bacteria exert selective pressure on other microorganisms in the same habitat as well. The existence of these resistance genes, as well as precursor proteins that originally have alternative biochemical functions but can easily change into resistance proteins in the case of selective pressure (Wright, 2007), are considered the source of resistance in human and animal bacterial populations (Allen et al., 2010; Chee-Sanford et al., 2009; D'Costa et al., 2007; D'Costa et al., 2006; Wright, 2007). The underlying idea is that bacteria excreted by humans and animals acquire these genes through horizontal gene transfer, resulting in bacteria resistant to antibiotics not (yet) seen previously in human or animal bacterial isolates. When these bacteria are subsequently re-introduced in humans or animals that are treated with antibiotics this may contribute to the quick development of resistance. The existence of natural resistance genes is demonstrated by the presence of low frequencies of resistance genes in *E. coli* isolated prior to

Table 10 Resistance against third generation cephalosporins in *E. coli* from food animals in different countries.

Country (year)	% resistant isolates (N isolates)				Reference
	Poultry products			Pork	
	C. jejuni	*C. coli*	*Campylobacter* spp.	*C. coli*	
Netherlands (2008)	57.1 (359)	55.6 (45)	-	-	(MARAN-2008)
Belgium (2008)	19.8 (111)	80.8 (73)		31.4 (70)	(EFSA, 2009c)
Denmark (2008)	19.2* (26)	-	-	-	(Jensen and Hammerum, 2009)
Germany (2008)	-	-	31.3 (15)	-	(EFSA, 2009c)
Latvia (2008)	100 (23)	-	-	-	(EFSA, 2009c)
Portugal (2008)	100 (37)	100 (25)	-	-	(EFSA, 2009c)
Spain (2008)	-	-	82.5 (193)	-	(EFSA, 2009c)
USA (2007)	17.2 (332)	25.9 (143)	-	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18125
Canada (2008)	0-10.8** (234)	0-16.7** (31)	-	-	(Anonymus, 2008)

* domestic cases
**depending on province or region

Table 11 Methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence in food samples in different countries.

Country (year)	% MRSA pos	Nsamples	Remarks	References
Netherlands (2006)	2,.5	79	Samples of various meat products from pigs and cattle (pork, n = 64; beef, n = 15)	(Van Loo et al., 2007)
Netherlands (2008)	11,9	2217	84% of MRSA ST398; number of MRSA bacteria very low (mostly <10 colony-forming units/gr)	(De Boer et al., 2009)
Italy (2003-2005)	0,4	1634	MRSA detected in samples from bovine milk (n=4) and dairy cheese products (n=2)	(Normanno et al., 2007)
Portugal (2006-2008)	-	not specified	0.68% (1/148) of the *S. aureus* strains isolated from food samples showed the presence of the gene *mecA* (from bovine mastitis)	(Pereira et al., 2009)
Spain (2007-2009)	1,6	318	ST398 was detected in food samples from pork and veal.	(Lozano et al., 2009)
Canada(2008)	7,7	402	Three major types were obtained: ST398 (30%), ST8 (40%) and ST5 (30%). ST8 has also been found frequently in horses, which may represent a source of contamination of meat. ST5 is a strain commonly found in humans in both the USA and Canada.	(Weese et al., 2009)
USA (2008)	5,0	120	Types found were USA100 (ST5) and USA300 (ST8)	(Pu et al., 2009)
Japan (2002-2003)	0,5	444	MRSA strains possessing SCC*mec* type IV, (a genotypic characteristic prevalent in community acquired-MRSA isolates), isolated from retail samples of chicken meat	(Kitai et al., 2005)
Jordan (2005)	1,2	1260	Meat samples from sheep, bovines, camels and poultry from Amman area were examined. MRSA was mainly detected in sheep and chicken meat.	(Quddoumi et al., 2006)
Korea (2001-2003)	0,2	930	Three pre-MRSA (SCCmec type III) and one silent *mecA*-carrying methicillin-susceptible S. aureus (smMSSA) were isolated from retail chicken meat	(Kwon et al., 2006)
Korea (2007)	-	not specified	Of the 133 staphylococci isolated from raw meats, 18% of the isolates were found to be resistant to methicillin, but none of these isolates showed the presence of the *mecA* gene	(Lee et al., 2008)

Table 12 Resistance against third generation cephalosporins in E. coli isolated from food samples in different countries.

E. coli		% resistant isolates (N)				
Country (year)	**Antimicrobial tested (breakpoint used)**	**Pork**	**Beef**	**Chicken meat**	**Turkey**	**Reference**
Netherlands(2008)	cefotaxime (>0,25 mg/l)	0 (26)	1.3 (75)	14.6 (329)	-	(MARAN-2008)
Denmark(2008)	cefotaxime(>0,25 mg/l)	0 (66)	0 (63)	0.9 (113)	-	(Jensen and Hammerum, 2009)
France (2008)	cefotaxime(>0,25 mg/l)	1.0 (102)	-	2.5 (240)	0.8 (246)	(EFSA, 2009c)
Germany (2008)	ceftazidime (>2 mg/l)	0 (1)	0	0 (2)	-	(EFSA, 2009c)
Spain (2008)	cefotaxime(>0,25 mg/l)	-	0 (10)	-	-	(EFSA, 2009c)
Sweden (2008)	ceftiofur (>4 mg/l)	0 (19)	-	-	-	(Bengtsson et al., 2009)
Canada (2008)	cefotaxime (>0,25 mg/l)	0-7.1* (318)	0.7-2.3* (572)	18.3-48.6* (480)	-	(Anonymus, 2008)
	ceftiofur (>4mg/l)	0-0.6* (318)	0-0.8* (572)	0 (480)	0 (29)	
USA (2007)	ceftriaxone(>32 mg/l)	0.7 (152)	0.8 (256)	6.0 (299)	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18127
	ceftiofur (>8 mg/l)	0 (152)	0 (256)	0.3 (299)	-	

* depending on province or region

Table 13 Resistance against third generation cephalosporins in Salmonella isolated from food samples in different countries.

Salmonella		% resistant isolates (Nisolates)				
Country (year)	**Cephalosporin tested (breakpoint used)**	**Pork**	**Beef**	**Chicken meat**	**Turkey**	**Reference**
Netherlands(2008)	cefotaxime (>0,5 mg/l)	13.0 (31)	9.0 (22)	15.3 (248)	-	(EFSA, 2009c)
Belgium (2008)	cefotaxime (>0,5 mg/l)	1.9 (159)	0 (17)	21.6 (218)		(EFSA, 2009c)
Denmark(2008)	cefotaxime (>0,5 mg/l)	1.0 (99)	-	-	-	(EFSA, 2009c)
France(2008)	cefotaxime (>0,5 mg/l)	-	-	-	-	(EFSA, 2009c)
Germany(2008)	ceftazidime (>2 mg/l)	-	-	-	-	(EFSA, 2009c)
Italy(2008)	cefotaxime (>0,5 mg/l)	0 (124)	0 (15)	2.7 (37)	0 (62)	(EFSA, 2009c)
Poland (2008)	cefotaxime (>0,5 mg/l)	-	-	0 (128)	0 (28)	(EFSA, 2009c)
Spain(2008)	cefotaxime (>0,5 mg/l)	0 (34)	0 (5)	0 (42)	3.0 (33)	(EFSA, 2009c)
Switzerland(2008)	ceftiofur (>4 mg/l)	-	-	0 (10)	-	(EFSA, 2009c)
Canada(2008)	ceftiofur(>4mg/l)	-	-	4.7-23.4* (382)	-	(Anonymus, 2008)
	ceftriaxone (>32 mg/l)			0-0.8* (382)	-	
USA (2008)	ceftiofur (>8 mg/l)	-	-	16.2 (99)		
	ceftriaxone (>32 mg/l)	0 (18)	0 (13)	0 (99)	-	http://www.ars.usda.gov//Main/site_main.htm?docid=18127

* depending on province or region

1950, before antibiotics were widely used (Hughes and Datta, 1983; Smith, 1967), and the presence of antibiotic resistance in pristine environments (Allen et al., 2009; Dib et al., 2008).

The use of antimicrobial drugs in human and animal healthcare, as well as additional applications such as aquaculture, crop protection[1], animal feed additives[2], and food conservation[3] has resulted in the widespread development of resistance not only in humans and animals, but also in the environmental reservoir.

Antibiotic-resistant bacteria and antibiotic resistance (AR) genes are excreted into the environment with faeces of humans and animals that are treated with antibiotics. Resistant bacteria and AR genes enter the environment through discharge of untreated or partially treated wastewater from hospitals, farms or slaughterhouses, sewage overflows during heavy rainfall, application of activated sludge from waste water treatment plants as fertilizer to agricultural soil, and run off of animal manure or faeces of pasture animals. Once in the environment, bacteria of different origin come into physical contact and

may exchange resistance genes with the endogenous bacterial population through a process called horizontal gene transfer (Coughter and Stewart, 1989; Cattoir et al., 2008; Genthner et al., 1988; Xu et al., 2007). Even though the resistant bacteria of human and animal origin may die off in the environment, the endogenous environmental bacteria may pass the acquired resistance genes on to their progeny. Despite the generally believed negative impact of acquired antibiotic resistance on fitness (Andersson and Levin, 1999; Andersson and Hughes, 2010), these genes may remain present in the environment for a long time (Andersson, 2003). Firstly, the presence of trace amounts of antibiotics in the environment, originating from treated humans and animals or from soil bacteria, may exert selective pressure. But also in the absence of antibiotics AR genes may be stable in the environment, due to: compensatory mutations that restore fitness, the existence of AR genes that do not decrease fitness or even increase fitness, the existence of alternative functions of the resistance protein in certain environments, such as the removal of toxic molecules by efflux pumps, and co-selection of the AR resistance genes with other genes on the same mobile genetic element that increase fitness in certain environments, such as the presence of heavy metals (Allen et al., 2010; Chee-Sanford et al., 2009; Pallecchi et al., 2008; Zhang et al., 2009). Thus, the environmental bacteria represent a long-lasting reservoir of AR genes, fed by bacteria that are secreted by man and animal.

3.4.2 Additional reservoirs

Next to humans, livestock and the environment a few additional reservoirs of antibiotic resistance have been identified. These reservoirs can be divided into three categories: reservoirs in which antibiotic resistance develops due to administration of antibiotics to cure or prevent disease (e.g. pets, zoo animals, ornamental fish, fish for consumption grown in tanks); reservoirs that pick up antibiotic resistance from the environment (e.g. wild life, fish cultivated in natural waters); and reservoirs to which bacteria and genes are introduced actively, and antibiotic resistance may be introduced as accidental or unwanted byproduct (e.g. fermented food and genetically modified food). These examples are illustrated below.

Companion animals, zoo animals and ornamental fish

There is evidence that resistance is increasing in bacteria of clinical importance in companion animals, particularly in pathogenic staphylococci. These animals can act as reservoirs of such bacteria for humans, in particular for their owners and veterinary staff (Lloyd, 2007). Also zoo animals were identified as a potential reservoir of antimicrobial-resistant bacteria and clinically important resistance genes in Japan (Ahmed et al., 2007). Antimicrobials are used to control bacterial infections in ornamental fish and are routinely added to the water during transport. Ornamental fish and their carriage water have been found to act as a reservoir for multidrug resistant bacteria and resistance genes (Verner-Jeffreys et al., 2009).

Fish, molluscs and crustaceans for consumption

Antibiotics are widely used in aquaculture, e.g. for the production of salmon, trout, and tropical seafood (Grave et al., 2008; Kumar et al., 2005). In the Netherlands, eel and catfish farms were identified as major reservoirs of oxytetracycline resistance genes, which might form a risk for human health (Penders and Stobberingh, 2008). Fish that are caught from natural waters and molluscs (that are cultured in natural waters), may be contaminated with antibiotic-resistant bacteria from the environmental reservoir. The presence of indicator bacteria has been demonstrated in molluscs (Al-Jebouri and Trollope, 1984), and given the presence of antibiotic-resistant bacteria in lakes and marine waters, these bacteria might carry antibiotic resistance, as was demonstrated for different types of bacteria isolated from battlenose dolphins (Schaefer et al., 2009).

Wild-life

Human commensal and pathogenic bacteria with antibiotic resistance have been detected in wild animals, such as rodents (Gilliver et al., 1999), wild boar (Poeta et al., 2007; Poeta et al., 2009), primates (Rolland et al., 1985; Rwego et al., 2008), marine mammals (Schaefer et al., 2009), and wild birds (Blanco et al., 2009; Bonnedahl et al., 2009; Cole et al., 2005; Dolejska et al., 2007; Dolejska et al., 2009; Hudson et al., 2000; Livermore et al., 2001; Waldenstrom et al., 2005). Since these animals do not receive antibiotics and direct contact with human or animal carriers of antibiotic-resistant bacteria is not likely, this finding suggest uptake of resistance from the environment. Through wild life antibiotic resistance can spread geographically, even to remote areas where antibiotics are not available (Gilliver et al., 1999; Sjölund et al., 2008).

Food associated bacteria and genetically modified (GM) crops

Bacteria deliberately added to the food chain or being an integral part of the food, such as lactic acid producing bacteria in starter cultures or other bacteria used to produce fermented foods (e.g. meat and cheese) and probiotics have been found to carry resistance genes and are therefore identified as potential reservoirs of antimicrobial resistance (EFSA, 2007). Another application is the use of antibiotic resistance markers as tools in plant biotechnology. The genetically modified plants are a potential reservoir of resistance genes, however, the risk of compromising the efficacy of antimicrobial drugs is

considered to be vanishingly small (EFB, 2001; EFSA, 2006). Nevertheless, public concerns on the use of GM crops with antibiotic resistance markers are widespread.

Notes

1 Antimicrobial use for crop protection purposes (high-value fruit, vegetables, and ornamental plants) has been phased out in the EU and only streptomycin usage is allowed in exceptional and well controlled and monitored circumstances for a short period (EC, 2009). In contrast, its usage is more widespread in fruit production in the USA, in particular for the control of fire blight of pome fruits (Rezzonico et al., 2009).

2 Growth promoters have been banned in the EU since 2006, but are still frequently used in e.g. the USA, Australia and South America.

3 Antibiotics were used in food preservation of meat, milk, canned foods, fruit and vegetables in the 1950s and 1960s but are now either restricted or prohibited (Sofos and Busta, 1992). Note that other (chemical) food processing techniques still in use may promote the generation and transfer of antimicrobial resistance (EFSA, 2007).

4 Transmission of antimicrobial resistance to humans

In this chapter, evidence of associations between antimicrobial resistance in food animals and humans (section 4.1) and the various transmission routes by which antimicrobial-resistant bacteria and antimicrobial resistance genes may be transmitted from food animals to humans (section 4.2) are summarized. Finally, the relative contribution of the food animal reservoir to resistance in humans is discussed (section 4.3).

4.1 Associations between antimicrobial resistance in food animals and humans

Associations between antimicrobial usage in human medicine and antimicrobial resistance in humans are evident and have frequently been reported (Goossens, 2009; Van de Sande-Bruinsma et al., 2008). Evidence of associations between antimicrobial usage and resistance in food animals and resistance in humans, however, is only fragmentarily available. For certain zoonotic bacteria, especially *Campylobacter* and *Salmonella* spp., there are numerous reports describing an association between resistance in human clinical isolates and antimicrobial usage and resistance in food animals. Also, there are several studies demonstrating an association between the occurrence of resistant commensal bacteria, especially enterococci and *E. coli*, in food animals and the (healthy) human population. However, the number of studies demonstrating an association between resistance among commensal bacteria in food animals and resistance in human clinical isolates is only limited.

Several lines of evidence for associations between antimicrobial usage and resistance in animals and

resistance in humans can be distinguished including (FAO/OIE/WHO, 2003):

- temporal and geographic associations;
- associations based on molecular characterization of strains;
- associations found in outbreak investigations;
- associations established in epidemiological (case-control) studies;
- associations found in field or experimental studies;
- associations established in modeling studies.

Temporal and geographic associations

- In several European countries, the introduction of apramycin, an aminoglycoside antibiotic, for veterinary use in the beginning of the 1980s induced the emergence of apramycin resistance among *E. coli* and *Salmonella* isolates from food animals and among human clinical isolates of *E. coli*, *Salmonella* and *Klebsiella* (Chaslus-Dancla et al., 1991; Johnson et al., 1995; Pohl et al., 1993; Wray et al., 1986).
- In several countries, including the Netherlands, there was a marked increase in quinolone resistance in human *Campylobacter* infections following the introduction of fluoroquinolones in food animals in the late 1980s (see *Campylobacter* below for more details).
- In Australia, where the use of fluoroquinolones in food animals was never approved, domestically acquired *Campylobacter* infections are susceptible to fluoroquinolones, whereas in European and North-American countries that allow the use of fluoroquinolones *Campylobacter* infections are commonly caused by fluoroquinolone-resistant *Campylobacter* (Unicomb et al., 2003). Also, fluoroquinolone resistance is becoming increasingly prevalent among human clinical *E. coli* isolates in the USA, whereas the prevalence of fluoroquinolone-resistance among human clinical *E. coli* isolates in Australia is very low (Collignon and Angulo, 2006).
- In Europe, the use of avoparcin – a vancomycin-like glycopeptide – as a growth promoter in food animal production caused a large increase of vancomycin-resistant enterococci (VRE) (Goldstein et al., 1993) in both food animals and the human population (Van den Bogaard and Stobberingh, 2000; Wegener et al., 1999). In contrast, in the USA, where avoparcin was never allowed for use in food animals, VRE have not been found in food animals or healthy people (Bonten et al., 2001). Since the introduction of VRE into the hospital was feared and especially the transfer of the glycopeptide resistance genes to MRSA (for which vancomycin is the drug of last resort), the use of avoparcin in food animals was banned in the EU from 1997. To what extent the increase of VRE in the human population has contributed to the increase in the prevalence of VRE in hospitals in several European countries (since 2000) is uncertain.
- In the Netherlands, LA-MRSA CC398 has emerged in hospitals since 2003 and simultaneously, this specific clone has spread through the Dutch food animal production sector (see LA-MRSA below for more details).
- Following a voluntary withdrawal of ceftiofur in chicken hatcheries in Canada in 2005, a significant decrease in ceftiofur resistance was observed in bacteria from retail chicken and humans (see ESBL below for more details).

Associations by molecular characterization

- Molecular characterization of the VanA transposons of vancomycin-resistant enterococci (Goldstein et al., 1993) isolated from (both healthy and hospitalized) humans and food animals in the Netherlands revealed identical Tn1546 types (Willems et al., 1999). Further molecular characterization revealed four distinct genogroups associated with particular hosts and environments (Willems et al., 2000). However, overlap was found between strains of poultry farmers and slaughterhouse workers and poultry and between strains of veal farmers and veal calves, suggesting direct transmission from food animals to humans. A genetic relationship was found between faecal isolates from both healthy and hospitalized humans and pigs in the Netherlands (Bruinsma et al., 2002). However, extensive molecular typing and comparative genomic hybridizations also revealed that invasive isolates recovered from hospitalized patients or clones associated with hospital-outbreaks were genetically distinct from human and animal surveillance (faeces) isolates (Willems et al., 2005).This demonstrates that VRE clones selected in the community are not responsible for infections and outbreaks in hospitals.
- Genetic similarity was found between isolates of gentamicin-resistant enterococci from humans, retail food and food animals in the USA, suggesting transmission from food animals to humans through the food supply. The emergence of gentamicin-resistant enterococci has been attributed to the use of gentamicin in food animals (Donabedian et al., 2003).
- In various case-studies, homology was found between LA-MRSA strains from humans and food animals (see LA-MRSA below for more details).
- In different studies, genetically indistinguishable plasmids carrying ESBL genes were detected in isolates from humans and food animals (see ESBLs below for more details).

Associations in outbreak investigations

In the past decade, numerous outbreaks of salmonellosis caused by resistant *Salmonella* strains have been associated with the consumption of foods of animal origin. In several cases a link was established with the occurrence of resistant *Salmonella* strains or antimicrobial usage in food animals. Some examples are given below:

- In the Netherlands, in 2003, a large increase in the incidence of salmonellosis caused by quinolone (nalidixic acid)-resistant S. Enteritidis phagetype 1 was associated with the import of eggs from Spain due to the avian influenza outbreak in Dutch poultry (Van Pelt et al., 2004). A relatively high percentage of Spanish eggs was found to be contaminated with nalidixic-resistant S. Enteritidis PT1 (Elson, 2004).
- In 2005, a ten-fold increase in multidrug resistant *Salmonella* Typhimurium DT104 cases in the Netherlands was associated with beef imported from a third country. The incriminated beef yielded S. Typhimurium DT104 of the outbreak-associated molecular type (Kivi et al., 2007).
- In the USA, outbreaks of multidrug resistant *Salmonella* Newport have been associated with consumption of foods of animal origin, including beef and cheeses made from nonpasteurized milk, as well as exposure to dairy farms (Gupta et al., 2003).
- In the UK, an outbreak of quinolone (nalidixic-acid)-resistant *Salmonella* Typhimurium DT104 was traced back to a dairy farm where fluoroquinolones were used in the dairy cattle in the month prior to the outbreak (Walker et al., 2000).

Epidemiological associations (case-control sporadic cases)

In several case-control studies, sporadic cases of antimicrobial-resistant infections in humans have been associated with the consumption of foods of animal origin. Some examples are given below:

- In the USA, sporadic cases of domestically acquired fluoroquinolone-resistant *Campylobacter* infections have been associated with the consumption of (domestically produced) poultry (Kassenborg et al., 2004).
- In another USA study, sporadic cases of multidrug resistant *Salmonella* Newport were associated with consumption of undercooked beef or scrambled eggs (Varma et al., 2006).
- In a Canadian study, urinary tract infections (UTI) in women caused by resistant *E. coli* were associated with consumption of foods of animal origin (see ESBL below for more details).
- In several studies, carriage of LA-MRSA was associated with exposure to food animals (see LA-MRSA below for more details).

Associations in field or experimental studies

- In experimental field studies in the 1970s, it was demonstrated that the addition of tetracycline to chicken feed induced the emergence of tetracycline resistance both in the chickens and in people living on the farm (Levy et al., 1976).
- In the former German Democratic Republic, the effects of the introduction of nourseothricin, a streptothricin antimicrobial agent that had not been used before in human or veterinary medicine, into pig production as a growth promoter was studied. The introduction induced the emergence of nourseothricin-resistant *E. coli* in pigs as well as in employees of the pig farms (Hummel et al., 1986). Subsequently, nourseothricin resistance was also detected in human *Salmonella* and Shigella isolates indicating horizontal transfer of the nourseothricin-resistant genes within the human intestinal flora (Witte et al., 2000).
- In an experimental study, treatment of individually housed broilers with fluoroquinolones quickly selected for high frequencies of quinolone-resistant *Campylobacter jejuni* strains (Van Boven et al., 2003). Poultry meat is considered a major source of human campylobacteriosis (see section 4.2.2).

Associations in modeling studies

- In a Danish study, using a mathematical model, the vast majority of human infections with antimicrobial-resistant *Salmonella* were attributed to (Danish or imported) foods of animal origin (Hald et al., 2007).

Quinolone-resistant *Campylobacter jejuni*

- After the introduction of enrofloxacin (a fluoroquinolone) for use in poultry and veal calves in 1987, an immediate increase of quinolone resistance in *Campylobacter* isolates from poultry and from human infections was observed. The increase in humans preceeded the introduction of ciprofloxacin in human medicine providing evidence of an association with the (extensive) use of fluoroquinolones in the poultry industry. The prevalence of quinolone-resistant *Campylobacter* isolates from poultry products increased from 0% to 14% in 1989 and the prevalence in humans increased from 0% to 11% 1989 (Endtz et al., 1991). Similar temporal associations between the first approved use of fluoroquinolones in food animals and an increase in fluoroquinolone-resistance in human *Campylobacter* infections were observed in other European countries and in the USA.
- As mentioned above, there is a clear geographic association between the occurrence of (domestically acquired) fluoroquinolone-resistant *Campylobacter* infections in humans and the use of fluoroquinolones in food animals.
- In several epidemiological studies, sporadic cases of antimicrobial-resistant *Campylobacter jejuni* infections in humans have been associated with the consumption of foods of animal origin, especially poultry (Engberg et al., 2004; Kassenborg et al., 2004; Painter et al., 2002).

LA-MRSA

- In the Netherlands, LA-MRSA CC398 has emerged in hospitals since 2003 and has increased towards 42% of human S. aureus isolates in 2008 (Haenen et al., 2010).

Simultaneously, this specific clone has spread through the Dutch food animal production sector (Wagenaar and Van de Giessen, 2009) (temporal association).
- The geographic distribution of LA-MRSA CC398 isolates from patients, clustering in the eastern and southern parts of the Netherlands, corresponds to the density of intensive livestock farming, whereas the distribution of non-CC398-MRSA isolates corresponds to the density of the Dutch human population (Van Loo et al., 2007).
- The vast majority of LA-MRSA-isolates from humans and of MRSA-isolates from food animals concern multilocus sequence type ST398. Moreover, the predominant Spa-types of MRSA ST398-isolates from food animals in the Netherlands are also the main types of LA-MRSA in the Dutch human population providing a clear genetic link. Also, in several case-studies indistinguishable types of LA-MRSA were found in pig farmers or their family members and in their pig herds (Lewis et al., 2008; Van Hoecke et al., 2009; Voss et al., 2005), Characteristically, almost all LA-MRSA-isolates in the Netherlands, both from food animals and humans, are resistant against tetracycline which antibiotic is frequently used in food animal production in the Netherlands (Wagenaar and Van de Giessen, 2009).
- In a case-control study among Dutch patients carriage of LA-MRSA was associated with exposure to pigs or cattle (Van Loo et al., 2007). In a Danish study, carriage of LA-MRSA was associated with living or working on farms with animals (Lewis et al., 2008). In Dutch on-farm studies, an increased risk of LA-MRSA carriage was found for people living or working on pig farms or veal farms. Moreover, in these studies carriage of LA-MRSA was asssociated with the intensity of contact with pigs or veal calves (Van den Broek et al., 2009; Wagenaar and Van de Giessen, 2009). In studies on pig and broiler slaughterhouses carriage of LA-MRSA among employees was associated with contact with live slaughter animals (Mulders et al., 2010; Van Cleef et al., 2010b).

ESBL-producing bacteria

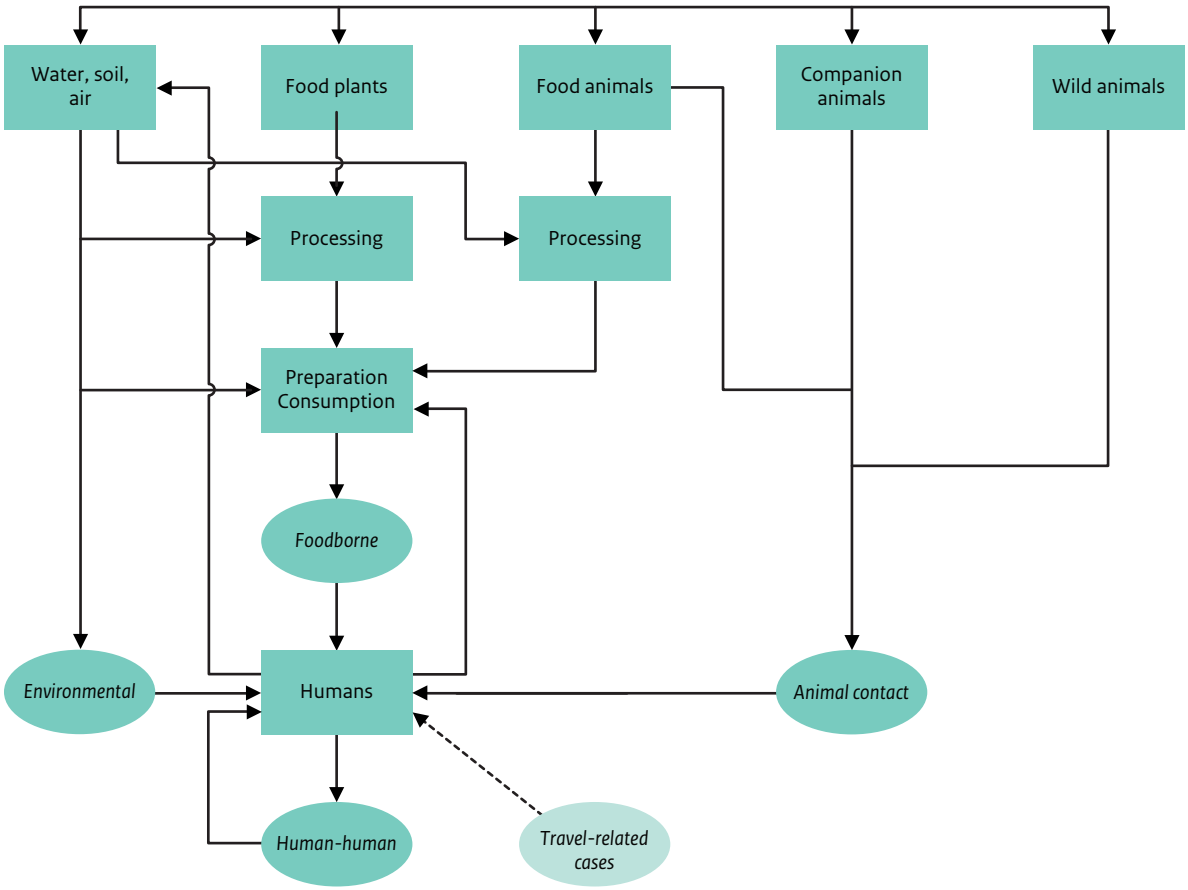
- Since the late 1990s, new ESBL genes of the cefotaximease family, CTX-M, emerged to become the predominant ESBL-type in human health care worldwide (Livermore et al., 2007). In Dutch hospitals, since 2001, an increase in the proportion of resistance to third generation cephalosporins of invasive *E. coli* isolates has been observed; in 2008 5% of invasive *E. coli* isolates recorded in the national surveillance system were non-susceptible to third-generation cephalosporins (SWAB. NethMap, 2009). Simultaneously, a significant increase in resistance against cefotaxime has been observed in *E. coli* isolates from broilers (temporal association). In broiler breeder production, ceftiofur (a cephalosporin) is used off-label in one-day-old chicks or in-ovo in combination with vaccination (Mevius, 2009).
- Following a voluntary withdrawal of ceftiofur in chicken hatcheries in Canada in 2005, a significant decrease in ceftiofur resistance was observed in *E. coli* and *Salmonella* Heidelberg isolates from retail chicken as well as in *Salmonella* Heidelberg isolates from humans. After reintroduction of use, increasing levels of extended-spectrum cephalosporin resistance were observed in bacteria from chicken and humans providing evidence of a temporal association (Dutil et al., 2010).
- Genetically indistinguishable plasmids carrying ESBL genes were detected in strains of *Salmonella* Bareilly isolated from poultry and human patients in the Netherlands and similarly, in strains of *Salmonella* Blockley from Dutch poultry, poultry meat and human patients (Hasman et al., 2005).
- In a recent Dutch study, 13% of the ESBL-genes and plasmids in human ESBL-producing clinical isolates were genetically related to genes and plasmids from Dutch poultry (D. Mevius, personal communication).
- In a Danish study, genetically indistinguishable or closely related plasmids carrying ESBL genes were detected in animal, human and environmental samples of two pig farms (Moodley and Guardabassi, 2009).
- In a Canadian case-control study, urinary tract infections (UTI) in women caused by multidrug-resistant *E. coli* were associated with chicken consumption, while UTI in women caused by ampicillin- or cephalosporin-resistant *E. coli* were associated with consumption of pork (Manges et al., 2007).

Strength of the associations

The evidence of the associations described above was evaluated and assessed qualitatively by using an approach for classification based on the system applied by the American Institute of Medicine (IOM, 2007). By using this method the strength of the associations can be classified as follows:

- **Sufficient evidence of a causal relationship:** the evidence is sufficient to conclude that there is a causal relationship between resistance in human clinical isolates and resistance in food animals. Several independent reports on either epidemiological studies, outbreak investigations or molecular characterization studies show consistent associations. In addition, the association is biologically plausible and the causality is corroborated by a temporal or geographic association or by experimental data.
- **Sufficient evidence of an association:** the evidence is sufficient to conclude that there is an association. Some independent reports on either epidemiological studies, outbreak investigations or molecular characterization studies show consistent associations. However, the number of these reports is only limited, the association is not biologically plausible or the causality is not corroborated by a temporal or

Figure 33 Major transmission routes of antimicrobial-resistant pathogens (EFSA, 2008a).



geographic association or by experimental data.
- **Limited or suggestive evidence of an asssociation:** the evidence suggests an association but the amount of evidence is limited.

Based on this classification method, it is concluded that:
- for quinolone-resistant *Campylobacter jejuni* as well as for LA-MRSA there is sufficient evidence of a causal relationship between resistance in human clinical isolates and resistance in food animals.
- for ESBLs there is sufficient evidence to conclude that there is an association between resistance in human clinical isolates and resistance in poultry. However, the number of studies showing consistent molecular or epidemiological associations is at present too small to conclude that there definitely is a causal relationship.

4.2 Transmission routes

Antimicrobial-resistant bacteria and resistance genes may be transferred from food animals to the general population via direct contact (section 4.2.1), by foods of animal origin (section 4.2.2), and through environmental routes (section 4.2.3). A schematic overview of the potential transmission routes is presented in Figure 33. A more detailed description of the specific transmission pathways is given in the respective sections.

The assumed main transmission routes from food animals to humans and the main food animal reservoirs of the four selected hazards of animal origin, are summarized in Table 14. Evidence of transmission through a particular route can be obtained from case-studies, outbreak investigations, molecular typing, case-control studies and surveillance data. Comprehensive data on the transmission routes of the respective hazards are provided in sections 4.2.1-4.2.4.

4.2.1 Transmission through direct contact with food animals

Antimicrobial-resistant bacteria can be transmitted to humans by direct physical contact with animals, e.g. petting, scratching and licking, and inhalation of air with contaminated dust/aerosols in the direct vicinity of the animal. In addition, transmission through physical contact with the contaminated animal environment (e.g. pen, fencing) and contaminated fomites (e.g. manure, clothing, equipment) may occur.

Table 14 The main identified food animal reservoirs and transmission routes from food animals to humans of the four selected antimicrobial resistance hazards.

	Food animal reservoirs	Main transmission route*
LA-MRSA	Pigs, veal calves, and broilers	Direct contact
Quinolone-resistant *Campylobacter jejuni*	Poultry, pigs, veal calves, and dairy cows	Food of animal origin
ESBL+ *Escherichia coli*	Broilers, veal calves, pigs, and dairy cows	Food of animal origin and/or direct contact
ESBL+ *Salmonella* spp.	Poultry	Food of animal origin

* Important to realize that the transmission is not restricted to these indicated routes.

Quinolone-resistant *Campylobacter jejuni*

Poultry, in particular broilers, is the main food animal reservoir of quinolone-resistant *Campylobacter jejuni*, but this bacterium is also found in the faeces of pigs, veal calves and dairy cows (MARAN-2007). Direct transmission from food animals to humans is assumed to follow faecal-oral routes.

Data on quinolone-resistant *C. jejuni* transmission through direct contact is scarce. Few case-control studies on quinolone-resistant *C. jejuni* infections have been published (Engberg et al., 2004; Evans et al., 2009; Johnson et al., 2008; Kassenborg et al., 2004; Painter et al., 2002); neither study found 'animal contact' to be a significant risk factor in multivariate analysis of the data. No indications of quinolone-resistant *C. jejuni* transmission through direct contact were found from case studies, outbreaks or surveillance data.

For susceptible *C. jejuni*, the attribution of direct contact was estimated to be of significant importance next to foodborne transmission (Evers et al., 2004), but with a large uncertainty. In a few studies, exposure to farm animals was found to be associated with campylobacteriosis (Friedman et al., 2004; Studahl and Andersson, 2000).

LA-MRSA

LA-MRSA is found in the nose, throat, faeces and on the skin of food animals (pigs, veal calves, and broilers) as well as in dust and air in the direct environment of these animals (Wagenaar and Van de Giessen, 2009). Possible transmission routes are direct skin-hand or faeces-hand pathways as well as more indirect transmission e.g. skin-dust-nose or faeces-pen-hand pathways.

Direct contact with pigs, veal calves and broilers is the main risk factor for LA-MRSA carriage (Mulders et al., 2010; Van Cleef et al., 2010a; Van Cleef et al., 2010b; Wagenaar and Van de Giessen, 2009) underlining the importance of this transmission route. Entering the animal houses and the intensity (duration) of contact with food animals is associated with increased risk of LA-MRSA carriage (Van den Broek et al., 2009; Wagenaar and Van de Giessen, 2009). The risk of specific farm activities was investigated on veal calves farms, but the data were too strongly correlated to be analyzed separately (Wagenaar and Van de Giessen, 2009). A number of reported cases of LA-MRSA infections concerned pig farmers or family members that were infected with similar LA-MRSA-types as found in their pig herds (Lewis et al., 2008; Van Hoecke et al., 2009), which indicates transmission through animal contact. However, the type of activity or contact that lead to infection was not identified, except in one case of a pig bite that lead to a severe wound infection (Declercq et al., 2008). It was found that LA-MRSA does not spread from farms into the community (Van Cleef et al., 2010a).

Working in the dirty area of a pig slaughterhouse is a risk factor, but no specific activity in the slaughterhouse proved to be significant (Van Cleef et al., 2010b). In poultry slaughterhouses, employees hanging broilers on the slaughterline have an increased risk of MRSA carriage, in particular when conventional electric stunning is used. It was hypothesized that, compared to CO_2 stunning, conventional electric stunning causes more dust in the slaughterhouse environment as a consequence of the extensive flapping of the broilers' wings. Contaminated dust and air are possible vehicles of LA-MRSA transmission from food animals to humans (Wagenaar and Van de Giessen, 2009).

ESBL-producing *Escherichia coli* and *Salmonella*

The main food animal reservoir of ESBL-producing Escherichia coli in the Netherlands is broiler production, but isolates were also found in faecal samples of veal calves, pigs and dairy cows (MARAN-2008). Poultry is the sole food animal reservoir of ESBL-producing *Salmonella* thus far (MARAN-2008).Transmission of *E. coli* and *Salmonella* by direct contact is assumed to mainly follow faecal-oral routes, after which the ESBL genes may possibly be transferred to human enteric bacteria.

Information on transmission of ESBL-producing *E. coli* and *Salmonella* by direct contact is scarce. Recently, strong indications that direct contact with food animals may lead to transfer of ESBL genes were found in a Danish study where indistinguishable or closely related plasmids carrying ESBL genes were detected in *E. coli* in animal, human and environmental samples of two pig farms (Moodley and Guardabassi, 2009). ESBL-producing *E. coli* was also detected in air (Moodley and Guardabassi, 2009) and various floor samples (Liebana et al., 2006) and dust in Irish broiler houses was found positive for ESBL-producing

Table 15 Food categories of animal origin based on EFSA (MARAN-2008) and main transmission pathways of foodborne bacteria based on Roberts (Roberts, 1990).

Main food categories of animal origin	Transmission pathways
Milk and dairy products	Contamination of the milk with bacteria of animal origin and consumption of raw milk or soft cheeses made of raw milk.
Eggs and egg products	Transovarial transfer of bacteria to egg contents and consumption of raw eggs.
Red meats	Faecal contamination of the carcass during the slaughter process and consumption of raw or inadequately heated meat or cross contamination during food preparation.
Poultry meats	Faecal contamination of the carcass during the slaughter process and consumption of inadequately heated meat or cross contamination during food preparation.
Aquaculture and marine	Fish: faecal contamination of the aquatic environment and consumption of raw fish. Shell fish: faecal contamination of the aquatic environment, concentration of bacteria in shell fish through filterfeeding, and consumption of raw or inadequately heated shell fish.
Mixed or buffet meals	One of the above mentioned pathways and consumption of inadequately stored/ heated meals.

Salmonella (Boyle et al., 2010), but the importance of transmission through air or dust is unclear.
No indications of transmission of ESBL-producing *E. coli* or *Salmonella* by direct contact were found from case-control studies, case studies, outbreaks or surveillance data. Similar non-ESBL-producing drug-resistant *E. coli* were found in farm animals and farmers (Katsunuma et al., 2008; Van den Bogaard et al., 2001), indicating that clonal spread by direct contact between animals and humans may occur. For non-ESBL-producing *Salmonella*, most risk factors are related to foodborne transmission; no associations with occupational exposure or ownership of farm animals were found in the Netherlands (Doorduyn et al., 2006). Only sporadic cases of *Salmonella* transmission through direct contact are known (Fey et al., 2000; Hendriksen et al., 2004) and one study showed the association of multidrug resistant *Salmonella* to direct exposure on dairy farms (Gupta et al., 2003). This implies that this transmission route is of minor importance for *Salmonella*.

4.2.2 Transmission through foods of animal origin

Food of animal origin is an important vehicle for the transmission of many zoonotic bacteria (including the resistant ones), e.g. *Salmonella* and *Campylobacter*. Also for commensal bacteria, food is likely to contribute to resistance transfer, as resistance genes are often located on mobile genetic elements and the high density of bacteria in the gut is beneficial for gene transfer.
Transmission may occur through handling or consumption of contaminated food products to which both the general population as well as food handlers may be exposed. Moreover, food handlers may be a reservoir themselves and cause foodborne outbreaks among the general population. To what extent people are exposed to food products contaminated with antimicrobial-resistant bacteria, depends on many factors during food processing, transport and preparation that may increase and reduce the bacterial load and on hygiene measures taken. Cross contamination may transmit antimicrobial-resistant bacteria from one food to another via hands, surfaces, equipment, etcetera. The main food categories of animal origin and the consumption associated transmission pathways are summarized in Table 15.

Quinolone-resistant *Campylobacter jejuni*

Quinolone-resistant *C. jejuni* is found in poultry raw meat products (MARAN-2007). Potential foodborne transmission routes are surface-to-hand transmission during food handling and consumption of contaminated meat products.
Quinolone-resistant *C. jejuni* is detected throughout the meat production chain: in food animals at the farm, in food animals at the slaughterhouse, and in retail meat (MARAN-2008). Quinolone-resistant *C. jejuni* was also found on packaging of raw meat in the UK, which could potentially cross-contaminate ready-to-eat foods during and after purchase (Burgess et al., 2005).
Occupational exposure to raw meat, was not found to be a risk factor in the quinolone-resistant case-control studies (Engberg et al., 2004; Evans et al., 2009; Johnson et al., 2008; Kassenborg et al., 2004; Painter et al., 2002). In contrast, occupational exposure to raw meat e.g. as a cook or butcher was associated with (susceptible) campylobacteriosis in the Netherlands (Doorduyn unpublished results), which was also previously reported (Adak et al., 1995).
In some case-control studies, consumption of meat is found to be a risk factor for quinolone-resistant *C. jejuni* infections: consumption of chicken by travel associated-cases and consumption of pre-cooked (undefined) cold meats (Painter et al., 2002), eating poultry outside of the home (Kassenborg et al., 2004), and eating fresh poultry other than chicken and turkey (Engberg et al., 2004).
Improper preparation of poultry in restaurants is

suggested to increase the risk of eating poultry outside the home (Kassenborg et al., 2004), but whether preparation errors are underlying the risks found is not investigated. Other studies did not find associations with meat consumption (Evans et al., 2009; Johnson et al., 2008). Regional differences in antimicrobial use in food animal production and hence in resistance levels in meat may partly explain differences in case-control studies found (Engberg et al., 2004; Ledergerber et al., 2003; MARAN-2007; Painter et al., 2002). Outbreaks of *C. jejuni* are rarely reported; one foodborne outbreak of quinolone-resistant *C. jejuni* identified undercooked chicken liver paté as the most likely source of infection, emphasizing the risks of improper food preparation (Forbes et al., 2009).

LA-MRSA

In the Netherlands, presence of LA-MRSA was demonstrated in red meats and poultry meats (De Boer et al., 2009). Potential foodborne transmission routes are surface-to-hand transmission during food handling and consumption of contaminated meat products.
LA-MRSA is detected throughout the meat production chain; in broilers and pigs after slaughter (Mulders et al., 2010; Van Cleef et al., 2010b), in meat samples during meat processing (De Jonge, personal communication) and on meat in retail (De Boer et al., 2009; Van Loo et al., 2007). Cross contamination has not been investigated.
Studies on employees of broiler and pig slaughterhouses showed that employees that did not have contact with live animals had a low, but slightly higher risk than the general Dutch population. Professionals handling raw meat in the meat processing industry and in institutional kitchens did not have a higher risk of MRSA carriage (De Jonge, personal communication). Hence, the risk to become carrier as a result of LA-MRSA transmission from contaminated meat to food handlers seems to be low.
There are no known LA-MRSA cases as a consequence of consumption or handling contaminated meat. Outbreaks of LA-MRSA have not been reported and are not likely to occur as *S. aureus* food poisoning is toxin-related and LA-MRSA rarely possesses toxin genes (Kluytmans, 2010). Foodborne outbreaks of MRSA are uncommon and rarely lead to invasive disease. A case of the latter has been described only once and concerned a fatal case of sepsis in a severely immunocompromised patient during a large hospital outbreak of MRSA (Kluytmans et al., 1995). The Food Safety Authority concluded that based on the information currently available, food plays no or only a negligible role in the spread of MRSA in the human population. Many of the data that are needed to fully substantiate this conclusion are not available. Further studies are needed to elucidate transmission routes of MRSA in relation to meat and other foods and to provide the tools for preventing the spread of MRSA (De Boer et al., 2009).

ESBL-producing *Escherichia coli* and *Salmonella*

ESBL-producing *E. coli* has been detected in poultry, pork, veal, beef, and lamb raw meat products in the Netherlands (Kluytmans, 2010; MARAN-2008). ESBL-producing *Salmonella* is detected in poultry raw meat products only (MARAN-2008). Transmission may occur through handling of raw meat products and consumption of contaminated meat products, possibly followed by ESBL gene transfer to bacteria in the human gastrointestinal tract.
Worldwide, ESBL-producing *E. coli* and *Salmonella* are detected throughout the meat production chain: in food animals at the farm, in food animals at the slaughterhouse, and in retail meat (Carattoli, 2008; Duan et al., 2006; Greko, 2009; Hasman et al., 2005; Jensen et al., 2006; Lavilla et al., 2008; MARAN-2008; Mesa et al., 2006a; Politi et al., 2005; Riaño et al., 2006; Smet et al., 2009; Warren et al., 2008).
Cross contamination during food processing has not been investigated for ESBL-producing *E. coli*, but is likely to occur since it is common for (tetracycline-resistant) *E. coli* (Wu et al., 2009) and (susceptible) *Salmonella* (Rasschaert et al., 2008).
No information related to transmission of ESBL-producing bacteria from contaminated food to food handlers was found. However, this transmission route cannot be excluded since occupational exposure to raw meat was found to be a risk factor for (susceptible) *Salmonella* infections (Doorduyn et al., 2006).
In an analysis of 132 acute gastroenteritis outbreaks in Spain, circumstantial evidence that meat can be a vector of ESBL-producing *E. coli* was provided (Lavilla et al., 2008). Both food handlers as well as food animals were probably involved as reservoirs for ESBL-producing *E. coli*. In another Spanish study, ESBL-producing *E. coli* was detected in salads, but not in cooked foods examined, emphasizing the importance of proper food preparation and cross contamination. Similarities between *E. coli* isolated from retail chicken and human urinary tract infections support the role of foodborne transmission (Vincent et al., 2010).
Information related to foodborne outbreaks of ESBL-producing *Salmonella* was not found. The ESBL suspected Salmonellas found in 2006/2007 in Dutch patients, partly originated from poultry, but the route of transmission for these cases is unknown (MARAN-2008). Antimicrobial susceptible *Salmonella* infections are mainly associated with contaminated food (Arlet et al., 2006; EFSA, 2008b).

4.2.3 Transmission through the environment

People are at risk of being exposed through the environment to antibiotic-resistant bacteria and AR genes originating from food animals. Exposure can occur for instance when people recreate in surface water contaminated with animal faeces, consume drinking water produced from unprotected sources, or when eating plant food that has been irrigated with faecally contaminated

surface- or ground water, fertilized using contaminated manure, or grown in contaminated soil.

Prevalence of antibiotic resistance in environmental compartments

Antibiotic-resistant bacteria and AR resistance genes associated with antibiotic use in animals have been detected in agricultural soil (Schmitt et al., 2006; Srinivasan et al., 2008), and in various aquatic environments on farms, such as waste-, surface-, ground- and drinking water (Caplin et al., 2008; Chee-Sanford et al., 2001; Kim et al., 2005; Koike et al., 2007). Antibiotic-resistant *Campylobacter* have been detected in abattoir drain water (Koenraad et al., 1995), which may subsequently be discharged (partially treated or untreated) onto surface water. Many other studies have demonstrated the presence of resistance genes (Zhang et al., 2009) and antibiotic-resistant bacteria in surface and ground water outside farmlands (Faria et al., 2009; Gallert et al., 2005; Goñi-Urriza et al., 2000; Hong et al., 2004; Servais and Passerat, 2009; Soge et al., 2009). In some of the mentioned studies, the source of the antibiotic-resistant bacteria and genes were likely at least partially of human origin, based on the presence of waste water treatment plants upstream of the sampling locations. However, the origin of antibiotic-resistant bacteria and resistance genes were not determined in these studies, and most waters likely contain a mix of bacteria from human and animal origin. The contribution of resistance originating from livestock will vary between waters at different locations, e.g. rural or urban. Results from a study performed at the RIVM in 2006 demonstrated that in an area with intensive husbandry in the Netherlands nearly half of *Escherichia coli* isolates and more than half of intestinal enterococci isolates from surface waters and sediments were resistant to one or more antibiotics (Blaak, 2010). Preliminary results from a more recent study (2008-2009) also indicate high prevalence of antibiotic resistance among these bacteria in the large Dutch rivers, Meuse, Rhine and New Meuse (Blaak et al., In preparation).

Quinolone-resistant *Campylobacter* spp.

The prevalence of *C. jejuni* and *C. coli* resistant to quinolones has increased enormously over the past couple of years in broiler chickens and veal calves in the Netherlands with frequencies as high as 60-80% and 40-70% respectively in 2006 and 2007 (MARAN-2007). *C. jejuni*, *C. coli* and *C. lari* are frequently detected in the environment, such as surface water, soil and wild birds (Brown et al., 2004; Denis et al., 2009a; Leatherbarrow et al., 2007). In the Netherlands, *C. coli* strains resistant to ciprofloxacin and nalidixic acid were isolated from surface waters in an area with intensive husbandry in Noord-Oost Brabant in 2006 (Blaak, 2010), and *Campylobacter* spp. resistant to these two antibiotics was isolated from the Meuse in January 2009 (Blaak et al., In preparation). Previously, quinolone-resistant *C. jejuni* and *C. coli* were also detected in sewage and poultry abbatoir waste water (Koenraad et al., 1995). Outside the Netherlands, quinolone-resistant *C. lari* strains were detected in soil, surface water and wild animals in a farming environment in the UK (Leatherbarrow et al., 2007).

MRSA

In the Netherlands, methicillin-resistant *S. aureus* variants, were detected in surface water in Noord-Oost Brabant (Blaak, 2010), an area with intensive husbandry, as well as in the Meuse and the Rhine (Blaak et al., In preparation). The variants isolated from the Meuse and the Rhine were confirmed to contain the *MecA*-gene. The source of these MRSA strains has not (yet) been investigated. The high density of pig farms in the east of Northern Brabant make it plausible that variants isolated from surface water in that area originate from animals and therefore represent LA-MRSA. The rivers Meuse and Rhine flow through urban as well as rural areas, and the detected MRSA strains could originate from human as well as from animal sources. Further studies are necessary to ascertain the prevalence of LA-MRSA in Dutch surface waters. Only a few other, foreign studies report on the presence of MRSA in the environment. MRSA has been detected at marine beaches in the USA (Soge et al., 2009). MRSA was also detected in activated sludge and effluent water from municipal wastewater treatment plants in Sweden, suggesting human origin of these variants (Borjesson et al., 2009a; Borjesson et al., 2009b).

ESBL-producing bacteria and ESBL-genes

Literature describing the presence of extended-spectrum beta-lactamase producing bacteria or ESBL-genes in environmental samples are scarce. However, given the increase of ESBL-producing *E. coli*, *Klebsiella Pneumoniae* and *Salmonella* spp. in animal husbandry (MARAN-2007) as well as health care centres (Sturm et al., 2009) in the Netherlands and in countries worldwide, the question to answer is not whether these bacteria are present in the environment, but rather at which frequency different ESBL-producing bacterial species are present in different environmental compartments. In the Netherlands, in 2006 4.5% of *E. coli* isolates originating from surface waters in Noord-Oost Brabant appeared resistant to cefotaxime with MIC-values >2 mg/L (Blaak, 2010), and are therefore suspected ESBL-producers (CDC, 2009). Between spring 2008 and spring 2009 5.6% of *E. coli* strains isolated from the Meuse were ESBL-producers (Blaak et al., In preparation). Outside the Netherlands, (suspected) ESBL-producing bacteria were found in surface-, drinking-, and waste water, as well as in wild animals, which are most likely obtained from the environmental reservoir

Table 16 Environmental E. coli, S. enteritidis, and K. pneumoniae carrying ESBL-or ESBL-related genes.

ESBL*	Third or fourth generation cephalosporine-resistant**	Gene family	Bacterial species or genus	Environmental compartment	Country	Reference
+	+ (CAD)	CTX-M, TEM, SHV	*E. coli*	Wild animals (gulls)	France	(Bonnedahl et al., 2009)
+	+	CTX-M , TEM	*E. coli*	Wild animals (birds, deer, fox)	Portugal	(Costa et al., 2004; Costa et al., 2006)
+	+ (COT, CAZ)	CTX-M , TEM	*E. coli*	Wild animals	Portugal	(Costa et al., 2008)
+	+ (COT, CAZ, CEP)	nd	*E. coli*	Sewage	Spain	(Mesa et al., 2006a)
+	+ (CAX, CAZ, CEP)	nd	*K. pneumoniae*	Sewage sludge and effluent hospital sewage plant	Brazil	(Prado et al., 2008)
+	+ (COT, CAZ)	TEM, AmpC CMY, OXA	*E. coli*	River water	Korea	(Hong et al., 2004)
+	nd	TEM, CTX-M	*E. coli*	Sewage sludge	Austria	(Reinthaler et al.)
-	+ (CAX)	SHV	*S. enteritidis*	Drinking water supply system	Nepal	(Bhatta et al., 2007)
-	+ (CAZ)	TEM	*E. coli*	Drinking water	Turkey	(Alpay-Karaoglu et al., 2007)
nd	- (CAX)	SHV	*K. pneumoniae*	Farm drinking water	USA	(Kim et al., 2005)
nd	- (COT, CAZ)	TEM, SHV	*E. coli*, *K. pneumoniae*	Estuarine water	Portugal	(Henriques et al., 2006)

* ESBL-phenotype was confirmed (+) or not confirmed (-) using double-disk confirmation tests or screening plates. ** Isolates were resistant or not for one or more third/fourth generation cephalosporines and hence suspected ESBL-producers(+) or not (-); Indicated between brackets are the antibiotics that were investigated.. nd = not determined. CAX = ceftriaxone; COT=cefotaxime; CAZ = ceftazidime; CAD = cefadroxil; CEP = cefepime

(Table 16). Additionally, clinically relevant ESBL-genes, not linked to specific microorganisms, were detected in soil in the Netherlands (Knapp et al., 2010). The environment has been implicated as a reservoir of novel ESBL-genes, because of the detection of precursor genes or genes related to CTX-M-type ESBL-genes in remote, unspoiled soils (Allen et al., 2009), and in members of the genus *Kluyvera*, including environmental species (Humeniuk et al., 2002; Olson et al., 2005; Rossolini et al., 2008; Saladin et al., 2002).

4.2.3.1 Transmission through water, air and soil

Currently, the relative contribution of the environment to antibiotic resistance observed in humans is not clear. Data concerning the risks of exposure to antibiotic resistance through water, air or soil are lacking. Presumably, the risk of exposure to antibiotic-resistant bacteria in environmental compartments is considerably lower compared to direct contact with food animals or consumption of animal products, due to lower numbers of viable resistant bacteria in the environment. For instance, the risk of exposure to *Campylobacter* of unknown resistance profile via recreational water was estimated to be 33 and 56 times lower compared to consumption of food and direct contact, respectively (Evers et al., 2004). Considering the omnipresence of antibiotic resistance in the environment, exposure through the environment cannot beforehand be considered negligible. Given the role of the environment as repository of anthropogenic as well as natural resistance in which genes may be exchanged, people may be exposed to bacteria carrying novel resistance genes not seen previously in animal or man.

In the Netherlands, drinking water and recreational water legislation ensure a high microbiological quality. Nevertheless, exposure to antibiotic-resistant microorganisms and resistance genes through these routes is all but imaginary. Firstly, the monitoring of water quality is not a watertight system: the presence of microorganisms is not monitored frequently and might be subject to fluctuations in time. Also, water quality is based on the presence of faecal indicator microorganisms (*E. coli*, enterococci) only, and the prevalence of indicator microorganisms may not necessarily correlate with the presence of other species of pathogens or environmental bacteria that have different survival dynamics in water. Hence, a good water quality does not guarantee the absence of antibiotic-resistant microorganisms. Secondly, people may recreate in surface waters not specifically designated as recreational waters. High frequencies of antibiotic-resistant bacteria (32-48% of *E. coli* and enterococci) in the rivers Meuse and Rhine (Blaak

Table 17 Recent foodborne outbreaks associated with bacterial contaminated fresh produce.

Product	Bacterial species	Country	References
Spinach	*E. coli* (O157:H7)	USA	(Grant et al., 2008; MMWR, 2006; Wendel et al., 2009)
Fresh peppers, tomatoes	S. Saintpaul	USA	(MMWR, 2008)
Cantaloupe	S. Poona	Mexico, USA, Canada	(MMWR, 2002)
Cantaloupe	S. Saintpaul	Australia	(Munnoch et al., 2009)
Basil	S. Senftenberg	Europe, USA	(Pezzoli et al., 2008)
Ruccula salad	S. Thompson	Europe	(Nygard et al., 2008)
Alfalfa sprouts	S. Weltevreden	Europe	(Emberland et al., 2007)
Tomatoes	S. Braenderup	USA	(Gupta et al., 2007)
Orange Juice	S. Typhimurium	USA	(Jain et al., 2009)

et al., In preparation) suggest a realistic risk of exposure through recreation, the magnitude of which needs to be established. Finally, even in tap water AR-genes have been detected (Xi et al., 2009; Zhang et al., 2009). The presence of AR-genes in drinking water despite the application of treatment processes might be explained by the incomplete removal of resistance genes, or incomplete removal of resistant bacteria followed by regrowth in drinking water distribution systems (Xi et al., 2009). Currently, it is not known whether resistance genes are present in Dutch drinking water and in what concentrations.

4.2.3.2 Transmission through foods of plant origin

A possible consequence of the presence of antibiotic-resistant bacteria and resistance genes of animal origin in the environment may be that fruits and vegetables harbour these bacteria and genes as well. Besides exposure to natural antibiotic resistance genes present in environmental bacteria, fruits and vegetables may be exposed to antibiotic-resistant bacteria of animal origin through the use of animal manure, contaminated soil, or using contaminated water as irrigation water. Although part of the contaminating bacteria will become inactivated during the 'farm-to-fork' chain, in case of fresh products this chain is relatively short and pathogen inactivation rates may therefore be low. Additionally, some products such as raspberries can not be washed or treated adequately because they are too fragile.

Little is known regarding the presence of antibiotic-resistant bacteria on food of plant origin. However, ample research has demonstrated the association between foodborne outbreaks and the consumption of fresh produce contaminated with human enteric pathogens (Lynch et al., 2009). Salad vegetables, fresh herbs but also fruits have been implicated in outbreaks with enteric pathogens *Salmonella* spp. and *E. coli* O157:H7 (Table 17). Also *Campylobacter* spp. and *Staphylococcus* spp. have been detected on fresh vegetables obtained in retail markets (Abadias et al., 2008; Thunberg et al., 2002). The presence of pathogenic bacteria on fresh produce is not always due to contamination from the environment, i.e. at the farm. Contamination may also occur in later stages in the 'farm-to-fork' chain, for instance via hands of food handlers during harvesting, processing and retail, dependent on the effective application of good agricultural practices (GAP). Nevertheless these reports demonstrate that human pathogens can survive on food of plant origin and subsequently be transmitted to humans. Given the omnipresence of antibiotic resistance in the environment, it is very likely that part of the bacteria that people are exposed to through fresh produce are resistant to antibiotics. Exposure to and colonization with antibiotic-resistant non-pathogenic bacteria, may even occur more often than the reported outbreaks suggest, because these events will go unnoticed.

Most studies describing contamination of fresh produce with antibiotic-resistant bacteria specifically focused on, or only isolated, enterococci (Abriouel et al., 2008; Johnston and Jaykus, 2004; Johnston et al., 2006; Rodríguez et al., 2006). Resistances to different classes of antibiotics were found, among which quinolones, macrolides, streptogramins, aminoglycosides, tetracyclines and beta-lactams, on different types of produce. ESBL-genes have also been demonstrated on vegetables and fruit, associated with environmental bacterial species *Rahnella* spp. (Ruimy et al., 2009). In one study, the presence of *E. coli* and *Salmonella* spp. was investigated on cantaloupes, oranges and parsley, irrigation water and other possible sources of contamination in the farm-to-fork chain (Duffy et al., 2005). Both *Salmonella* and *E. coli* were mainly found on cantaloupes. One of the *Salmonella* serotypes found on cantaloupes (*S. javiana*) was also present in irrigation water, although Pulsed Field Gel Electroforese (PFGE) analysis could identify neither irrigation water nor processing equipment as contamination source. For the *Salmonella* strains antibiotic resistance profiles were determined: one of the *Salmonella* strains from cantaloupe was streptomycine-resistant, other isolates, among which isolates from irrigation water, were intermediately sensitive to this antibiotic.

4.3 Relative contribution of the food animal reservoir to resistance in humans

For over 40 years, there is a vivid debate on the contribution of the food animal reservoir and resistance in humans. The extent to which the food animal reservoir contributes to resistance in humans is the result of complex interactions of interrelated factors and is therefore difficult to quantify. Indications on the relative contribution of the food animal reservoir to resistance in humans can be obtained from microbiological approaches, epidemiological approaches, intervention studies and expert elicitation (Pires et al., 2009). For the selected hazards this information is given throughout this risk profile and is summarized below.

Quinolone-resistant *Campylobacter* spp.
In the Netherlands, approximately 50% of *Campylobacter* strains isolated from humans are resistant to fluoroquinolone; there is no information available on source attribution of these strains (MARAN-2008). Approximately 7% of the fluoroquinolone-resistant cases are travel related (MARAN-2008), which includes contribution of the (foreign) food animal reservoir. Microbial subtyping has shown that approximately 50 to 80% of all human cases of *Campylobacter jejuni* can be attributed to the chicken reservoir (EFSA, 2009d); no data on subtyping of fluoroquinolone resistant strains have yet been published.

LA-MRSA
Epidemiological studies indicate that approximately 30% of professionals that have direct contact with food animals are carriers of LA-MRSA and have similar subtypes as their food animals (Graveland et al., 2008; Van den Broek et al., 2009; Wagenaar and Van de Giessen, 2009). Person-to-person transmission is limited, which reduces the risk of secondary spread to family members (Van den Broek et al., 2009; Wagenaar and Van de Giessen, 2009), people living in the same municipality (Van Cleef et al., 2010a), and limits hospital outbreaks (Wassenberg et al., 2010). There are no indications that the presence of MRSA on meat is a risk for public health (De Boer et al., 2009) or for professionals handling food (R. de Jonge, personal communication). Annually, approximately 10-15% of all MRSA infections are attributed to LA-MRSA* (Haenen et al., 2009; Haenen et al., 2010).

ESBL-producing bacteria
In a pilot study, 6 out of 18 Dutch broiler farmers were found positive for ESBL-producers in their faeces (D. Mevius, personal communication). In a recent study conducted in the Netherlands, 13% of the ESBL-genes and plasmids in human ESBL-producing clinical isolates were genetically related to genes and plasmids from Dutch poultry (D. Mevius, personal communication). The attribution of the food animal reservoir to resistance in the general Dutch population is unknown.

Concluding remark
Depending on the bug-drug combination, there is a reasonable amount of information available that indicates that resistance in food animals contributes to resistance in humans. However, to what extent the food animal reservoir contributes to human resistance is still undetermined.

Note
* Note that these are presumed infections; the numbers are based on the number of MRSA isolates obtained from infection materials (blood, purulence, abscess and wound fluid).

5 Future hazards

Based on expert opinion the following future hazards and future consequences were anticipated.

Future hazards

Further emergence of antimicrobial resistance

The past 60 years of antimicrobial use have shown that antimicrobial resistance may evolve rapidly in response to clinical and agricultural use of antimicrobial drugs. Resistance genes may either develop from modifications of existing resistance genes or from cryptic resistance genes that hide undetected in the genomes of antibiotic-sensitive bacteria (Hall, 2004). The development of resistance to newly introduced antimicrobial drugs is difficult to predict and depends on many factors, including antimicrobial use policies (Hall, 2004). Every antibiotic so far discovered has conferred resistance. Most threatening is the development of new resistance mechanisms that involve resistance genes that are on mobile genetic elements and facilitates spread within and between species.

Persistence of multidrug resistance

As explained in section 2.1, antimicrobial resistance may persist even after withdrawal of the use of the antimicrobial drug. This may be caused by several mechanisms (Andersson, 2003) and (Zhang et al., 2006), amongst others genetic linkage to other resistances, i.e. co-resistance. The main threat is the persistence of multidrug resistance, which will make several classes of antimicrobial drugs permanently ineffective. An example is the presistance and even increase in the occurrence of resistance to Chloramphenicol in *E. coli* in food animals, while this drug had not been used in these animals for more than 15 years (MARAN 2008).

Evolution of existing strains and the emergence of new variants

Existing strains of food animal origin (e.g. LA-MRSA) may evolve and become more virulent, e.g. by acquisition of toxin genes or genes related to increased infectiousness or invasiveness. Moreover new (multidrug-resistant) variants may colonize animals under the high selection pressure of continuous antimicrobial drug use. These future strains may be more pathogenic and more adapted to human hosts, thereby posing a threat to public health.

Increased resistance in the environment

The majority of antimicrobial resistance genes have their origin in the environment, in particular soil bacteria that are an inexhaustible source of resistance genes. Contamination of the natural environment by the release of large amounts of antimicrobials, heavy metals or other pollutants may increase the selection pressure on antibiotic resistance in the environment. In addition, contamination with animal and human antibiotic-resistant commensal and pathogenic bacteria presumably results in uptake of antibiotic-resistance genes by environmental bacteria, and vice versa. Both phenomena may result in the introduction of new resistant organisms into both the

food animal and the human population (Allen et al., 2010; Martínez, 2008).

Influx of resistance from abroad

Influx of resistant bacteria and resistance genes from other countries into the Netherlands may occur through patients that were nursed abroad, travelers, emigrants, animals that were purchased abroad, imported food products, etcetera. In particular cross border patient mobility is frequent in the Euregion and has been implicated in the dissemination of resistant bacteria and resistance genes, e.g. MRSA (Deurenberg et al., 2009). Also the environment facilitates spread of antibiotic resistance across country borders, e.g. through rivers and streams, ballast water of ships (Drake et al., 2007), but even air and sand (Kellogg and Griffin, 2006), and via wild animals (Pallecchi et al., 2008). Especially migratory birds have been implicated in long distance spread of antibiotic resistance and transmission to people living in remote communities where antibiotics are not or only rarely used (Sjölund et al., 2008). These are ongoing processes that threaten the relatively low resistance levels in the Dutch health care system as well as public health. Strict rules concerning the treatment of patients that were nursed abroad (isolation and screening) are already in place. In the future it may lead to travel restrictions and, in the case of animals and food products, export problems (see 'Future consequences' below).

Future consequences

Suboptimal treatment of human and animal disease

A major consequence of antimicrobial resistance is the suboptimal treatment of human and animal disease. The main threat is the development of multidrug-resistant (MDR) bacteria that have become resistant to all available antimicrobial drugs, i.e. pandrug-resistant (PDR) bacteria. Currently, pandrug-resistant strains of Gram-negative bacteria are increasingly found in Europe, e.g. PDR *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Gould, 2008).

Export complications

Restricted import/export of animals and food products of animal origin contaminated with antimicrobial resistant bacteria may lead to high costs, reduced animal welfare, and reduced consumer confidence, as a result of screening of food products, the destruction of batches, and culling of flocks/herds. Until now, only the Danish Veterinary and Food Administration implemented a zero-tolerance policy for multidrug resistant variants of *Salmonella* Typhimurium DT104, including imported food products.

6 Possibilities for intervention

Options for intervention in the transmission of antimicrobial resistance from food animals to humans are based on two general principles:

1. Prevention of development and dissemination of antimicrobial resistance in food animals.
   This includes:
   a. prudent use of antimicrobial drugs;
   b. prevention of transmission of resistant bacteria between food animals and between farms.
2. Prevention of transmission of resistant bacteria from food animals to humans.
   This includes:
   a. hygiene measures to prevent transmission through direct contact;
   b. general 'farm-to-fork' hygiene measures to prevent foodborne transmission;
   c. measures to prevent transmission through the environment.

Intervention strategies need to be based on both principles to maximize their effectiveness. To evaluate the effectiveness of intervention strategies, surveillance of antimicrobial usage and resistance is essential.

Current control measures and their presumed effectiveness are described in section 6.1. Further possibilities for control are described in 6.2.

6.1 Current control measures in the food animal production branche

Use of antimicrobial drugs

Responsible and prudent use of antimicrobial drugs in veterinary medicine is of vital importance to protect both animal and human health. A guideline for responsible and prudent use has been developed by the Animal Health Organization (OIE), (Anthony et al., 2001).

The Dutch Royal Veterinary Association (KNMvD) has developed an antibiotics policy which included formularies to support veterinarians in their selection of antimicrobial drugs. The formularies are aimed to create conditions for optimal effectiveness and to prevent the emergence and spread of antibiotic-resistant bacteria from veterinary use (Van den Bogaard, 1993).

In December 2008, covenants between the Ministry of Agriculture, Nature and Food Quality (LNV, now EL&I) and the four main food-producing animal sectors were signed. The aim of these covenants is to realize a reduction of antibiotic resistance in food-producing animals through selective and restrictive use of antibiotics. The parties involved are the umbrella organizations of farmers, processing industry, feed industry and KNMvD. Each sector has developed a detailed plan and follows its own approach, but they all contain the following three general principles:

1. outline of responsibilities in prescription, sale and administration of antimicrobial drugs,

2. increase of transparency by monitoring of antimicrobial drugs use with appropriate control systems, and
3. implementation of research and communication strategies for prudent antibiotic usage.

All actions currently undertaken should result in a 20% reduction of antimicrobial drug use in 2011 and a reduction of 50% by 2013. As most plans have been implemented during 2009, it may take some time for effects to become fully visible. In Denmark, extensive control programs mainly aimed at reducing the use of antimicrobial drugs seem to be effective (Wegener, 2010).

Current measures to prevent transmission between food animals and between farms

Transmission of bacteria between food animals and between farms may be prevented by general biosecurity and hygiene measures of which many are part of the IKB (Integral Chain Control) quality system, and by specific control programmes. In the past two decennia, monitoring and control measures for *Salmonella* Enteritidis and *Salmonella* Typhimurium have been successfully implemented in the poultry meat and egg sector to control the spread of these serovars through the poultry production chain and to reduce their prevalence in the different poultry populations. For antimicrobial-resistant bacteria, there are no specific control measures in place, but the general hygiene measures are assumed to be as effective for resistant bacteria as for non-resistant bacteria.

Current measures to prevent transmission through direct contact

There has been some attention to occupational transmission of antimicrobial resistance, e.g. VRE and LA-MRSA carriage (Van den Bogaard et al., 2001; Wagenaar and Van de Giessen, 2009), but until now this has not led to specific measures other than general hygiene measures. The effectiveness of some personal protection measures has been evaluated for LA-MRSA, see section 6.2.

Current measures to prevent foodborne transmission

In the food processing industry, several general control measures, including the application of good manufacturing practices (GMP), hygiene codes and HACCP (hazard analysis and critical control points), have been taken to reduce bacterial contamination of end products and prevent transmission of foodborne pathogens to humans. Furthermore, educational activities have been conducted by the government and the Nutrition Centre to inform consumers about hygienic food preparation and proper storage of foods. These general measures are assumed to be as effective for resistant bacteria as for non-resistant bacteria.

Current measures to prevent transmission through the environment

Currently, there are no specific measures to prevent transmission of antimicrobial resistance trough the environment. General measures, such as waste water treatment, inactivation of animal manure before application onto the land, and restrictions regarding the use of activated sludge as fertilizer may reduce the influx of resistance into the environment. Furthermore, the transmission from the environment to the human population may be reduced by recreational and drinking water quality regulations. However, the effectiveness of these measures on transmission of antimicrobial resistance has not been studied.

Quinolone-resistant *Campylobacter* spp., LA-MRSA and ESBL-producing bacteria

Currently, there are no specific measures to prevent transmission of LA-MRSA, quinolone-resistant *Campylobacter* spp. or ESBL-producing bacteria among food animals and to humans. The recently implemented general prudent use measures as agreed by the covenants may prevent further dissemination of these resistant bacteria and eventually reduce their prevalence in food animals. The single specific prudent use measure at present is the voluntarily stop of the veterinarians to use cephalosporins in poultry, because of the increase of ESBL-producing bacteria in poultry and poultry meat. Results from a voluntary withdrawal of ceftiofur use in hatcheries in Canada indicate that this might be an effective measure to reduce the levels of ESBL-producing *Salmonella* and *E. coli* on poultry meat and reduce ESBL-producing *Salmonella* infections in humans (Dutil et al., 2010). The effectiveness on ESBL-producing *E. coli* infections and carriage in humans is unknown.

6.2 Further possibilities for control

Prudent use of antimicrobial drugs

Further possibilities to diminish the use of antibiotics in food animals have extensively been described in a recent white paper (Knowledge-Chain-Infectious-Diseases-of-Animals, 2010). In this white paper, several approaches and research lines aimed at eliminating the systematic use of antibiotics in the animal production sector are described, including amongst others:

- pathogen and disease free design of food animal production;
- the development and use of alternatives for antibiotics, including vaccines;
- improved diagnostic tools;
- health promoting nutrition and genetic approaches to enhance disease resistance;

- governance and behavioural aspects that affect prudent use of antibiotics.

Other prudent use measures include:

- selective use of narrow spectrum in stead of broad spectrum antibiotics;
- reducing exposure of animals to antibiotics through preventive group-treaments;
- restricting the use of critical antimicrobial drugs for human treatment in food animals. These restrictions should take into account the lists of critically important antimicrobials for human and veterinary health as provided by the WHO (WHO, 2007) and OIE (OIE, 2007) and should include future classes of antimicrobial drugs developed for human use;
- decoupling of prescription and sale of veterinary drugs by veterinarians, a control option that is part of the Danish antimicrobial resistance control programme, has been investigated for the Dutch situation (Berenschot, 2010). It was concluded that this control option would be effective, but only if additional measures are taken to prevent unwanted effects such as the illegal purchase of antimicrobial drugs and diminished quality of veterinary health care. These additional measures will be costly and should be weighed against other control options available.

Measures to prevent transmission between food animals and between farms

Future possibilities to prevent transmission between food animals and between farms have extensively been descibed in the recent white paper (Knowledge-Chain-Infectious-Diseases-of-Animals, 2010). Other possibilities are the further implementation of closed farming systems.

Measures to prevent transmission by direct contact

Measures to prevent transmission of resistant bacteria by direct contact with food animals should be aimed to minimize exposure of professionals to live animals and their environment at the farm, during transport and in the slaughterhouse. This could be achieved by:

- adaptations in animal production systems, or
- personal protective measures including the wearing of protective clothing such as masks and gloves These measures are theoretically effective, but if not properly applied may increase risk of colonization (see LA-MRSA). Therefore, education about the use of personal protective measures should be provided. In addition, the use of personal hygiene measures, such as hand-washing and shower facilities should be encouraged.

Measures to prevent foodborne transmission

Further possibilities to prevent foodborne transmission of antimicrobial resistance include:

- extension of current hygiene codes and HACCP systems in the food processing industry to cover specific resistant bacteria. In Denmark a zero-tolerance has been imposed for multidrug resistant *Salmonella* DT104 in food products (Wegener, 2010). The effectiveness of this measure is yet unclear;
- decontamination of food products, in particular meat, by (improved) physical, chemical, biological and combined treatments, e.g. by washing, steam, pressurized water, electrolyzed water, ozonated water, irradiation, freezing, ultrasound, air chilling or freezing (Loretz et al., 2010). Note that some decontamination treatments of food products, such as irradiation and chemical decontamination, are not generally accepted and have not been evaluated for antimicrobial resistance. Moreover, it is feared that any treatment that induces bacterial stress may result in increased bacterial tolerance to the decontaminants or to increased resistance to (therapeutic) antimicrobial agents. In 2008, the EFSA BIOHAZ Panel examined four substances used to treat poultry carcasses and found no indications that this is the case (EFSA, 2008b);
- improved decontamination of the meat industry environment and equipment, e.g. by using cold plasma techniques (Leipold et al., 2010);
- intensified education to consumers about proper food preparation practices.

Environmental measures

Further possibilities for control are:

- improvement of sewage treatment to reduce resistant bacteria and resistance genes in discharged effluents;
- separate treatment of large farm and slaughterhouse waste water on location;
- implementation of treatment options regarding the use of animal manure and activated sludge as fertilizer;
- quality control of irrigation water;
- including antibiotic resistance as parameter of quality control of recreational waters and drinking water.

Note that information with respect to the transmission to and exposure via the environment is scarce and the effectiveness of the possibilities mentioned is as yet unclear.

Quinolone-resistant *Campylobacter* spp.

The control of foodborne transmission of quinolone-resistant *Campylobacter* should be similar to that of susceptible *Campylobacter*. Various control strategies to reduce the burden of campylobacteriosis by foodborne transmission have been discussed by Wagenaar (Wagenaar et al., 2006). They conclude that preventive measures in primary production have a limited and unpredicted effect, and that alternatives, such as vaccination, competitive exclusion, bacteriophage therapy and addition of bacteriocins to feed, are still experimental. According to the authors, post-harvest measures, in particular

scheduled processing (i.e. to separate positive and negative flocks) followed by freezing or chemical decontamination of the meat from positive flocks, are most promising and cost-effective. More recent data suggests that *Campylobacter* may also be transmitted from primary production to humans through routes other than food (van Pelt et al., 2008b). This strengthens the attention on primary production for an effective intervention.
A ban on enrofloxacin use in poultry has been effective in the USA since 2005. Three studies with respect to the effectiveness of this control option have been executed (Han et al., 2009; Nannapaneni et al., 2009; Price et al., 2007a). Price et al. (2007a) and Nannapeneni et al.(2009) show that fluoroquinolone-resistant *Campylobacter* is persistent in the first years after the ban. Han et al. (2009) report a low prevalence and a low ciprofloxacin resistance rate in retail chicken after the ban, but due the lack of suitable pre-ban data, differences in resistance levels before and after the ban could not be demonstrated.

LA-MRSA

Purchase of animals from LA-MRSA-negative farms has been associated with a lower prevalence of LA-MRSA on pig farms indicating the need for an integrated chain approach to control the dissemination of LA-MRSA. Restrictive use of antibiotics has been associated with a lower prevalence of LA-MRSA in veal calves and, although not always statistically significant, there seems to be a relation between antimicrobial drug use and the occurrence of LA-MRSA on pig farms as well (Van Duijkeren et al., 2008; Wagenaar and Van de Giessen, 2009). The effectiveness of prudent use measures on the prevalence of LA-MRSA in food animals, however, is yet unclear and should be investigated. This also applies to thorough cleaning and disinfection procedures that have been associated with a lower prevalence of LA-MRSA in veal calves (Wagenaar and Van de Giessen, 2009).
Alternative options to control the spread of LA-MRSA among food animals, such as phagetherapy (Mann, 2008) and vaccination are still under investigation. So far, *S. aureus* vaccines were unsuccessful to prevent colonization in humans (Creech Ii et al., 2009).
To prevent transmission of LA-MRSA from food animals to humans, direct exposure of professionals to food animals and their environment should be reduced, both on livestock farms and during transport and in slaughterhouses. This may be achieved by adaptations in animal production systems, e.g. by housing systems that acquire less employees or less time spent in contact with the animals, or by introducing personal protection measures. However, the effectiveness of personal protection measures, such as the use of (disposable) protective clothing covering wounds and skin lesions, and personal hygiene measures, such as hand washing facilities, should be further looked into. The usefulness of masks, gowns and gloves, to minimize the exposure of professionals on pig farms is limited, unless they are consistently and properly applied (Nathaus, 2009; Wulf et al., 2008a), otherwise they may even increase the risk of colonization (Denis et al., 2009b). Cleaning and disinfection of contaminated environments with specific attention to dust should be looked into (Catry et al., 2010). In poultry slaughterhouses, transmission of LA-MRSA to employees working with live birds can be reduced by stunning of the broilers before hanging them at the slaughterline, thereby reducing exposure to dust (Mulders et al., 2010).

ESBL-producing bacteria

Besides prudent use, no further control options are mentioned in the literature, most likely because information on transmission routes is scarce. ESBL-producing bacteria are multidrug-resistant and co-resistance to other antimicrobial drugs, e.g. aminoglycosides and fluoroquinolones, has been found. Hence prudent use of all antimicrobial drugs may be an effective measure to reduce the prevalence of ESBL-producing bacteria and should be looked into. Besides prudent use, effective infection control measures to interfere with transmission of ESBL-producing *E. coli* bacteria in animal production chains and farms need to be considered.

7 Risk assessment

Antimicrobial resistance risk assessment (AMR-RA) is a scientific tool to qualitatively or quantitatively evaluate the health risk resulting from exposure to resistant bacteria or resistance genes. In this section, an overview on AMR-RA is given, the feasibility of AMR-RA for the selected hazards is evaluated, and data needs and knowledge gaps are identified.

Antimicrobial resistance risk assessments (AMR-RA) framework

Guidelines for foodborne AMR-RA are under development by the Codex Alimentarius Commission (Codex, 2009). An important first step before the start of a risk assessment is the formulation of a risk management question. The risk management question should specify the hazard and the related consequences to be investigated as well as other details such as the country, time period, source, population at risk, and sequela of interest. The risk question may consist of two parts, the frequency and consequences, for example:

1. What is the contribution of cephalosporin use in poultry production in the Netherlands in 2007-2008 to resistance of *E. coli* in uncomplicated urinary tract infections?
2. What is the probability of an adverse human health effect as a consequence of these infections?

AMR-RA follows a similar four-step framework as used in microbial risk assessment (Codex, 2009). This framework is specifically developed for foodborne transmission, but could serve as a guiding principle for AMR-RA related to transmission through direct contact or the environment.
Step one, hazard identification, aims to identify the antimicrobial resistance hazard. Note that the hazard in AMR-RA could be a resistant bacterium (zoonosis) as well as a resistance gene.
The second step, exposure assessment, identifies the pathways of exposure and summarizes the data for the specific pathways in order to estimate the frequency and amount of the hazard to which humans are exposed. It should consider all reservoirs (food animals, humans, pets, environmental reservoirs, etcetera), all possible pathways of the hazard to the population of interest, and, in the case of resistance genes, it should take into account the possible gene transfer to human pathogens. This makes AMR-RA very complex; the complexity may be reduced by focussing on the main transmission routes. If relevant, pathways can be compared with each other (comparative exposure assessment).
Hazard characterization, the third step, aims to determine the probability of disease as a consequence of exposure to the hazard. AMR-RA should include the additional consequences of resistance, such as increased frequency and severity of disease.
In the final step, risk characterization, the results of the preceding steps are integrated and an overall estimate of risk is generated. Risk characterization answers the original risk management question including uncertainties and other factors that may affect the risk management decision.

AMR-RA methodology

AMR-RA methodology is extensively described in various articles, e.g. Vose 2001 and Snary 2004. AMR-RA can either be quantitative or qualitative, depending on whether the output is expressed in qualitative terms or numerically. The quality of the output strongly depends on the data quality and availability. Important data limitations encountered in AMR-RA are summarized in Snary 2004, these are: low compatibility in resistance data and microbiological methods used, large variability in sampling methods, small sample sizes, little data on indicator organisms, test sensitivity/specificity lacking, uncertain on causality, limited data on antimicrobial drug use. The identification of data gaps is an important output of risk assessments that can be helpful in prioritizing future research and data collection. Data limitations can be overcome by the use of expert opinion, surrogate data, e.g. data on the susceptible organism of the same species, a related species or a different resistance gene, a later starting point in the risk pathway, and predictive mathematical modelling (Snary et al., 2004). There are various sources of uncertainty in AMR-RA data. Sensitivity analysis is an important tool by which the effect of uncertainty in the model input on the model output can be investigated. Some sources of uncertainty in AMR-RA are mentioned in Claycamp and Hooberman (Claycamp and Hooberman, 2004), among which: the numerous indirect pathways, the quantification of the transfer of resistance genes within the animal or human gut, and the possible large period of time between exposure and infection due to resistance in commensal bacteria.

Overview available AMR-RA in the literature

In Table 18, an overview of the available AMR-RA is given. A number of things stand out, namely that:

1. most AMR-RA focus on foodborne transmission as opposed to transmission via the environment or direct contact;
2. AMR-RA are being commissioned by both government and industry (and sometimes come to opposite conclusions);
3. there is a lot of mutual criticism on the methodology used, quality of the data and assumptions. Examples of such critical comments are summarized in Table 19.

Overview available AMR-RA for the selected health hazards

For the selected hazards, AMR-RAs are only available for quinolone-resistant *Campylobacter* spp.; the results are summarized below.

Anderson et al. (2001) estimated that after ten years of fluoroquinolone use in beef production in the USA, approximately 30% out of 950 hospitalized and 280 other campylobacteriosis cases related to beef consumption and seeking treatment have a fluoroquinolone-resistant infection and need additional therapy and/or longer hospitalization (worst case scenario 85%). Associated mortality was 1-3 deaths (out of 3-4 deaths) per year. Note that in contrast to the Netherlands, fluoroquinolones are the therapy of choice for human enteric infections in the USA.

The VLA risk assessment (2004) estimated that 59.6% of quinolone-resistant *Campylobacter* cases in the UK were travel related, less than 1.5% were related to treatment of human cases, 8% were related to consumption of chicken (1.3% domestic and 6.7% non-UK chicken), 3.6% to contact with dogs and cats, and pig meat consumption, public water consumption and crop consumption contributed to less than one case per year. Thirty percent of total cases were the result of other sources than considered in this risk assessment. Incremental health risks are not addressed in this assessment.

Vose et al. (2001a) estimated that in 1998 and 1999 on average 4.5 and 6.2% of USA citizens with fluoroquinolone-resistant campylobacteriosis cases attributed to chicken that seek care and are prescribed an antimicrobial were affected by the fluoroquinolone resistance. For the total population, this risk is estimated at 1 in 34,651 and 1 in 26,639 persons that were affected.

Cox and Popken (2006) assessed the positive (i.e. less foodborne pathogens transmitted) and negative human health impacts (treatment failure) of continued use of enrofloxacin (and macrolides) in poultry, expressed as a benefit:risk ratio (Cox Jr and Ricci, 2005). They estimated this ratio to be 703 for enrofloxacin and conclude that withdrawal of this drug will cause more human illness than it would prevent.

In summary: the available AMR-RA are quantitative, but are difficult to compare, because they all have different lines of approach, other types of models, different assumptions, and concern different (food animal) reservoirs and countries. All risk assessments show that (fluoro)quinolone use in the food animal reservoir contributes to resistance in humans with varying estimated frequencies. The impact on human health consequences seems to be relatively small.

Feasibility of AMR-RA for the selected hazards

The quality of the output of AMR-RA strongly depends on the availability and quality of data and is particularly useful when transmission includes various pathways in which increase and reduction of the bacterial load takes place. Methods that may support or offer alternatives for AMR-RA are microbiological approaches, epidemiological approaches, intervention studies and expert elicitation (Pires et al., 2009). The usefulness of AMR-RA and alternative methods for answering risk questions with respect to the three selected hazards is evaluated below.

There are increasing amounts of epidemiological data available for **LA-MRSA**. LA-MRSA has a clearly identified

Table 18 Overview of available AMR-RA evaluating the human health risk/source attribution of antimicrobial use in food animals (partly based on Snary (2004).

Organism	Resistance	Food animal reservoir	Funding	Qualitative/ Quantitative	Risk management question	Conclusion	Reference
Campylobacter spp.	Quinolone	Cattle	Industry	Quantitative	What is the potential public health risk from *Campylobacter jejuni* and fluoroquinolone-resistant *C. jejuni* because of fresh beef and ground beef consumption?	After 10 years of fluoroquinolone use in beef cattle, 280 individuals and 45 hospitalized cases need additional therapy and/or longer hospitalization, and there is one associated death.	(Anderson et al., 2001)
		Poultry	Government	Quantitative	What is the human health impact of fluoroquinolone-resistant *Campylobacter* in broilers in 1998/1999 in the USA?	In the total population, 1 in 34,651/26,639 persons were affected; 1 in 23/17 cases seeking care and prescribed an antibiotic were affected.	(Vose et al., 2001a; Bartholomew et al., 2005)
		Poultry and pigs	Government	Quantitative	What is the contribution of the food chain to the problem of quinolone resistance in microorganisms causing human infection relative to other pathways?	Attribution: exposure abroad (59.6%), chicken meat (8%), pets (3.6%), clinical treatment (1.5%). Pig meat, public water and crop consumption not significant.	(VLA, 2004)
	Macrolide	Poultry	NS	Qualitative	What is the risk of transmission of macrolide-resistant *Campylobacter* from pigs to man?	Transmission through pig meat and environment (slurry/ waste) has no or a very low risk to man.	(Burch, 2002)
		Poultry	Industry	Quantitative	How is food animal illness related to human foodborne disease? Example: macrolide resistance in *Campylobacter*.	The removal of macrolide feed additives in chickens could harm human health by increasing the level of contaminated chicken.	(Singer et al., 2007)
		Poultry, pigs and cattle	Government	Quantitative	What is the potential human health risk due to foodborne *Campylobacter* spp. Infections derived from on-farm macrolide use?	The human health risks due to macrolide-induced resistance in *Campylobacter* are extremely low.	(Hurd et al., 2008)

Organism	Resistance	Food animal reservoir	Funding	Qualitative/ Quantitative	Risk management question	Conclusion	Reference
	Macrolide/ Quinolone	Poultry	NS	Quantitative	What are the likely human health impacts of continuing versus withdrawing use of fluoroquinolones and macrolides in production of broiler chickens in the USA?	Withdrawing animal antimicrobials can cause many more human illness-days than it would prevent.	(Reinthaler et al.)
Salmonella spp.	Quinolone	Food animals	Govern-ment	Qualitative	What is the risk of adverse human health effects consequent upon the development of antimicrobial resistance to (fluoro) quinolones in *S. typhimurium* due to the veterinary use of (fluoro)quinolones.	The probability of adverse health effects in humans appears to be low, but has a high degree of uncertainty and with much variation by country and species of livestock.	(Woolridge, 1999)
	MDR	Pigs	Govern-ment	Quantitative	What is the risk for Danish consumers of different control strategies for Salmonella typhimurium DT104?	If continued trade is implemented, the consumer risk increases by 2-3 times.	(Sommer et al., 2003)
	MDR and Quinolone	Animal food sources	Govern-ment	Quantitative	What is the contribution of various animal-foods and travelling abroad as sources to human infections with resistant Salmonella	Infections with resistant isolates commonly related to imported food products and travelling.	(Hald et al., 2007)
Enterococ-cus spp.	Penicillins	Food animals	Industry	Quantitative	What is the risk from continued use of penicillin-based drugs in food animals in the USA?	Current penicillin usage in food animals in the USA presents very low (possibly zero) human health risks.	(Cox et al., 2009)
	Strepto-gramin	Poultry	NS	Quantitative	What is the potential human health risk from virginiamycine use in chickens in the USA and Australia?	Banning virginiamycine use in chickens would be expected to save less than one statistical life in five years in the USA and Australia.	(Cox Jr and Popken, 2004a)
			NS	Quantitative	What is the human health risk from viriginamycine use in food animals?	Health risks are very small ($<1.10^{6}$, <1 excess death).	(Cox Jr and Popken, 2004b)
			Industry	Quantitative	What factors affect the prevalence of human commensal antimicrobial-resistant bacteria that cause opportunistic infection?	Agricultural antimicrobials use hastens resistance and decreases the efficacy of antimicrobials in humans; the greatest impact occurs very early in the emergence of resistance.	(Smith et al., 2002)

Organism	Resistance	Food animal reservoir	Funding	Qualitative/ Quantitative	Risk management question	Conclusion	Reference
			Industry	Quantitative	What are the likely public health effects of banning virginiamycin as a growth promoter (including person-to-person transmission)?	Inconclusive. Benefits of a ban are highest for intermediate values of the epidemic potential.	(Kelly et al., 2004)
			Industry	Quantitative	What are the human health impacts from withdrawing virginiamycin?	A withdrawal would cause more human illness than it would prevent.	(Cox Jr, 2005)
Campylobacter spp. + *Enterococcus faecium*	Macrolide	Poultry, pigs and cattle	Industry	Quantitative	What is the human health risk attributable to consumption of contaminated poultry, porc or beef with macrolide-resistant *Campylobacter* spp. or *E. faecium* due to tylosin/tilmicosin use in food animals in the USA?	Tylosin and tilmicosin use in livestock presents a low risk (less than 1 in 10 million) and less than 1 in 3 billion for foodborne illness from *Campylobacter* spp. and *E. faecium*.	(Hurd et al., 2004)
Campylobacter spp. + *Enterococcus faecium* + *Escherichia coli*	Various classes	Poultry, pigs, cattle, veal calves	NS	Semi-quantitative (expert opinion, risk scoring)	What is the risk to consumers arising from the exposure to antimicrobial-resistant bacteria from meat of four different types and four different product categories at retail level?	Fresh and frozen chicken contributed 6.7% and fresh and dry pork meat products contributed 4%. The contribution of beef and veal was low (0.4 and 0.1%).	(Presi et al., 2009)
Escherichia coli + *Salmonella* spp.	Various classes	Pigs	Product board and government	Quantitative	What is the risk of transmission of bacterial resistance with regard to the production and consumption of pork?	The impact of the veterinary use of antimicrobials is limited.	(Berends et al., 2001)
Various human pathogens	NS	NS	Industry	Qualitative (expert opinion)	What is the contribution from animal sources to the overall antimicrobial resistance problem in humans?	The role played by animal use of antimicrobials as a source of resistance is perceived to be relatively minor.	(Bywater and Casewell, 2000)

NS = not specified

Table 19 Overview published comments on AMR-RA.

Reference	Criticized by	Comments
Vose et al. (2001a); Bartholomew et al. (2005)	Bailar and Travers (2002); Cox (2005);Phillips et al. (2004)*	Estimates only numbers and probabilities of various kinds of infections, but could be extended to other endpoints; Omission of microbial load (exposure) and omission of frequency and severity of human health harm; Assessment relies on unsupported assumptions, especially that human health harm is proportional to chicken consumption.
Cox and Popken (2006); Cox and Ricci (2005)	Claycamp (2006)	Value antimicrobial-resistant and antimicrobial-sensitive illness equally, ignore population differences in resistant and sensitive illness, only cite one article on the treatment of diarrheal illness, whereas alternative references are available, only emphasize factors that tend to increase the benefit-risk ratio.
Hurd et al. (2004)	Tollefson et al. (2004)	Uses inappropriate estimate of the drug use parameters, ignores horizontal gene transfer in enterococci, did not include cross-contamination events, estimates the percentage poultry carrying resistant *Campylobacter* too low, estimates percentage contaminated meat too low, gives probability of risk is given in risk per serving instead of annual risk.
Bywater and Casewell (2000)	Bailar and Travers (2002)	Lacks discussion on model weaknesses and uncertainties in output, lacks information on selection of experts and their expertise, and includes all aspects of ill-health in a single scale.

* This review is critizised by many others (e.g. Jensen et al., 2004b; Tollefson et al., 2004; Turnidge, 2004)

main transmission route and risk group (professionals with direct contact with live food animals), although other minor pathways cannot be excluded. For most risk questions on frequencies of carriers and evaluation of intervention options, analytical epidemiological studies combined with microbial subtyping may suffice. For more complicated questions on e.g. health consequences, AMR-RA may be considered.

Quinolone-resistant *Campylobacter* has various pathways of mainly foodborne transmission. There is detailed information available on (susceptible) *Campylobacter* and various risk assessments have been performed for both susceptible (Nauta, 2009) and resistant *Campylobacter* (see 'Overview available AMR-RA for the selected health hazards' above). Besides AMR-RA also microbial subtyping may be useful for risk questions with regard to source attribution. Due to the various pathways, analytical epidemiological studies are less useful.

ESBL-producing bacteria are assumed to spread through various pathways. However, with the current knowledge, risk questions are difficult to answer with AMR-RA because of the general lack of data on transmission mechanisms, health consequences, gene transfer information, etcetera. Moreover, gene and plasmid transfer makes the dynamics highly complex. Before AMR-RA can be performed more detailed information is needed. Risk questions on source attribution, may be answered with plasmid subtyping. Epidemiological studies are less useful due to the complexity of the various pathways and gene transfer, which makes it difficult to link human cases to the animal reservoir.

8 Answers to the questions and recommen-dations

Answers to the questions

The answers to the questions below are focused on the three selected hazards, i.e. LA-MRSA, quinolone-resistant *Campylobacter* and ESBL-producing bacteria.

1. *What are the adverse effects of antimicrobial resistance to human health care and public health and what is the magnitude of these effects?*

Antimicrobial resistance reduces the efficacy of initial empirical treatment and limits the choice of treatment after diagnosis. There is an increase in disease burden, economic costs, and social consequences, but to which extent is not yet known (see section 2.7).

It is estimated that in the Netherlands, approximately 79,000 symptomatic ***Campylobacter*** infections occur annually and approximately 50% of these infections, i.e. 39,500 cases, are caused by quinolone-resistant strains. There are no indications that the disease burden has increased as a consequence of quinolone resistance. The costs for healthcare of quinolone-resistant *Campylobacter* infections are similar to those for susceptible *Campylobacter* infections, as invasive infections are rare and no additional measures are needed due to the absence of person-to-person spread. Quinolones are no longer recommended for empirical treatment of gastroenteritis because of high quinolone resistance levels in *Campylobacter*, which limits treatment options. Social consequences have not been documented.

In the Netherlands, approximately 100 **LA-MRSA** infections are reported per year; no fatal cases are known. The majority of LA-MRSA infections are skin and soft tissue infections, but this MRSA type is also capable

of causing severe infections in humans, such as endocarditis. LA-MRSA is a multidrug resistant bacterium; treatment options are strongly reduced. There are currently no indications that the individual burden of LA-MRSA infections differs from other MRSA infections. Livestock has developed into an independent reservoir of MRSA. The economic consequences of LA-MRSA have not been investigated but in addition to the costs of treatment, there is a 15% increase in MRSA screenings and a 44% increase of detected carriers who need to be isolated in hospitals, which obviously leads to an increase in direct costs for healthcare. Studies on MRSA in healthcare settings indicate that, besides somatic problems, people do experience psychological problems as a consequence of feeling stigmatized and being isolated.

ESBL-producing bacteria mainly cause urinary tract infections (UTI), but are also associated with invasive infections. It is estimated that in 2009, 1% of the UTI in general practice patients (approximately 5400 cases) were caused by ESBL-producing *E. coli*. In addition, there are an estimated 500 invasive infections by ESBL-producing bacteria reported in 2009. ESBLs are an important reason for failure of initial treatment with cephalosporins; infections with ESBL-producing bacteria are associated with increased length of hospital stay, and increased morbidity and mortality. Screening of risk patients and isolation of positive patients is needed. Alternative treatment options for these multidrug resistant bacteria are limited, more expensive and may require hospitalization. The increased disease burden and economic consequences due to resistance have not yet been quantified, but are likely to be substantial. The social consequences have not been investigated.

2. *How strong is the evidence of an association between antimicrobial resistance in food animals and resistance in humans?*

For the zoonotic microorganisms *Campylobacter* spp. and *Salmonella enterica* there are numerous reports describing an association between resistant bacteria in humans and in food animals, similar to susceptible bacteria. Also, several studies demonstrate an association between resistant commensal bacteria, i.e. enterococci and *E. coli*, in food animals and the general human population. The number of studies that describe an association between resistance among commensal bacteria in food animals and human clinical isolates is limited (see section 4.1).

For **quinolone-resistant *Campylobacter jejuni*** there is sufficient evidence of a causal relationship between resistant bacteria in humans and in food animals, in particular poultry.

For **LA-MRSA**, there is sufficient evidence of a causal relationship between resistant bacteria in humans and in food animals, in particular pigs, veal calves and poultry.

For **ESBL-producing bacteria** there is sufficient evidence of an association between plasmids and the resistance genes they carry in human clinical isolates and in poultry isolates. The number of studies showing consistent molecular or epidemiological associations is at present too small to conclude that there is a causal relationship.

3. *Through which routes does transmission occur?*

Antimicrobial-resistant bacteria and resistance genes may be transmitted from food animals to humans through direct contact with these animals, through foods of animal origin, and through environmental routes (see section 4.2).

Quinolone-resistant *Campylobacter* is mainly transmitted through consumption and preparation of broiler meat. There are no indications that professional handling of meat and direct contact with food animals poses a significant risk. The importance of transmission through environmental pathways to humans is unclear.

LA-MRSA is transmitted from livestock, mainly pigs, veal calves and broilers, to directly exposed humans. Although LA-MRSA has been detected on raw meat in retail, there are no indications that foodborne transmission is a risk for consumers or for people handling meat. LA-MRSA doest not spread to people living in the direct vicinity of farms.

ESBL-producing bacteria, such as *Salmonella enterica* en *E. coli*, and ESBL-genes may be transmitted by direct contact with food animals and foodborne transmission, which includes contaminated meat and vegetables. The importance of transmission through environmental pathways to humans is unclear.

4. *To what extent does antimicrobial resistance in food animals contribute to resistance in humans?*

The extent to which the food animal reservoir contributes to resistance in humans is the result of complex interactions of interrelated factors and is therefore difficult to quantify (see section 4.3).

Microbial subtyping has shown that approximately 50 to 80% of all human cases of ***Campylobacter jejuni*** can be attributed to the chicken reservoir (EFSA, 2009); no data on subtyping of fluoroquinolone-resistant strains have yet been published.

In the past few years, approximately 10-15% of all reported MRSA infections were caused by **LA-MRSA** and thus can be

attributed to the food animal reservoir. Approximately 30% of professionals that have direct contact with food animals, in particular pigs and veal calves, are carriers of LA-MRSA. Person-to-person transmission is limited, which reduces the risk of secondary spread, including hospital outbreaks.

In a recent preliminary study conducted in the Netherlands, 13% of the **ESBL**-genes and plasmids in human ESBL-producing clinical isolates were genetically related to genes and plasmids from Dutch poultry and in a pilot study, 6 out of 18 Dutch broiler farmers were found positive for ESBL-producing bacteria in their faeces. The attribution of the food animal reservoir to resistance in the general Dutch population is unknown.

5. *What are the options for intervention and what is their presumed effectiveness?*

Options for intervention may be based on two general principles:
1. prevention of development and dissemination of antimicrobial resistance in food animals;
2. prevention of transmission of resistant bacteria from food animals to humans.

Intervention strategies should consider both approaches to maximize effectiveness (see chapter 6).

Control of **quinolone-resistant *Campylobacter*** is similar to control of susceptible *Campylobacter* and may include post-harvest measures to prevent foodborne transmission. Improved slaughter hygiene as well as scheduled processing combined with decontamination measures are effective and efficient control options for both sensitive and resistant *Campylobacter*. The ban on the use of enrofloxacin in poultry in the USA did not result in reduced fluoroquinolone resistance in *Campylobacter* in the first years after the ban.

Purchase of MRSA-negative animals, restrictive use of antimicrobials and thorough cleaning and disinfection procedures have been associated with a lower prevalence of **LA-MRSA** in pigs or veal calves, but the effect of these measures is yet unclear. LA-MRSA exposure of professionals on livestock farms and slaughterhouses to live farm animals and their environment can be reduced by adaptations in animal production systems or by introducing personal protection measures such as the wearing of masks and gloves. These measures are theoretically effective, but if not properly applied may increase the risk of colonization.

Control options for the multidrug-resistant **ESBL-producing bacteria** are only scantily mentioned in the literature, but may include prudent use measures. In Canada, a temporary stop on using cephalosporins in poultry was effective in reducing the prevalence of ESBL-producing bacteria on poultry meat.

6. *Which future hazards can be anticipated?*

The following anticipated future hazards were identified (see chapter 5):
- *Further emergence of antimicrobial resistance*
  Antimicrobial resistance evolves extremely rapidly in response to clinical and agricultural use of antimicrobial drugs. Particularly the emergence of resistance based on genes located on mobile genetic elements is worrisome, as it can spread within and between species. If the prevelance of carbapenemases increases in human, their introduction in (food) animals can be expected.
- *Persistence of multidrug resistance*
  Multidrug resistance caused by genetically linked resistance genes on mobile genetic elements will make several classes of antimicrobial drugs permanently ineffective. Moreover, this phenomenon will result in persistence of multidrug-resistant isolates through co-selection by several antimicrobial classes.
- *Evolution of existing resistant strains*
  Existing resistant bacteria of food animal origin (e.g. LA-MRSA) may evolve and become more virulent and/or more adapted to human hosts, increasing the threat to public health.
- *Resistance in the environment*
  The environment is the natural reservoir of antimicrobial resistance genes. Contamination by antimicrobials and pollutants from animal husbandry, and the occurrence of human and animal bacteria in the environment may increase the selection pressure in the environment and cause the introduction of new resistant organisms into both the food animal and the human population.
- Influx of resistance
  Influx of resistant bacteria and resistance genes from foreign countries by patients, travelers, animal transports, imported food products, surface water, et cetera, are ongoing processes that threaten the Dutch health care system as well as public health.

The following future consequences of resistance development were identified:
- *Suboptimal treatment of human and animal disease*
  A major consequence of antimicrobial resistance is treatment failure, which will result in increased disease burden, economic costs and will affect everyday life of patients and their families. If the resistance levels increase over a certain threshold, standard empiric therapy protocols need to be changed to last resort drugs (e.g. carbapenems and vancomycin). This may result in selection of pandrug-resistant bacteria.

Table 20 The main identified knowledge gaps for quinolone-resistant Campylobacter, LA-MRSA, and ESBL-producing bacteria in the Netherlands (data availability: + high, +/- medium, - low).

	Quinolone-resistant *Campylobacter*	LA-MRSA	ESBL-producing bacteria
Populations at risk	+ (susc. *Campylobacter*)	+	-
Type and severity of adverse health effects	+ (susc. *Campylobacter*)	+/-	-
Disease burden	+ (susc. *Campylobacter*)	-	-
Economic consequences	+ (susc. *Campylobacter*)	-	-
Social consequences	-	+/-	-
Transmission routes	+/-	+	-
Contribution of the food animal reservoir to resistance in humans	+/-	+	+/-*
Intervention possibilities	+/- (susc. *Campylobacter*)	+/-	-

* Situation in the general population is not clear

- *Export problems*
  Possible future restrictions of import/export of animals and food products contaminated with antimicrobial-resistant bacteria may have economical consequences.

Recommendations

Monitoring of human cases attributed to the food animal reservoir

The effectiveness of the various prudent use measures that are (to be) taken in food animal production will be evaluated on antimicrobial usage and antimicrobial resistance in food animals and food of animal origin. These measures however, are mainly aimed to reduce current and future human health problems related to resistance from the food animal reservoir and should therefore also be evaluated on human health. It is therefore recommended to refine or make better use of the current human surveillance systems to enable monitoring of microbial resistance hazards attributed to the food animal reservoir.

Knowledge gaps

In this risk profile, numerous knowledge gaps were identified for the three selected hazards. The main data gaps with respect to the transmission of these resistant bacteria from food animals to humans and the consequences for human health are summarized in Table 20. These data are needed to conduct risk assessments and to evaluate risk management options. It is therefore recommended that research should be initiated to fill in these data gaps and that risk assessors should be involved when determining national research agendas, e.g. on the ESBL problem.

Antimicrobial resistance risk assessment (AMR-RA)

The usefulness of AMR-RA and other source attribution approaches for answering risk questions for the three selected hazards is evaluated in chapter 7. It is recommended that AMR-RA is used for answering *Campylobacter* risk questions and possibly for more complicated LA-MRSA risk questions, e.g. with respect to health consequences. AMR-RAs are considered useful for ESBL-producing bacteria as well. However, risk questions are currently difficult to answer because of the general lack of data on transmission mechanisms, gene transfer, et cetera, and will be very complex due to gene and plasmid transfer.

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Appendices

List of abbreviations

ABRES-vet-med:	Project on the transmission of antimicrobial resistance from food animals to humans and the consequences for health care and public health resulting in this risk profile
ADD:	Animal Daily Dose
AmpC:	Type of beta-lactamase
AMR-RA:	AntiMicrobial Resistance Risk Assessment
ATC:	Anatomical Therapeutic Chemical classification system
BRMO:	Multidrug-resistant micro-organisms
CA-MRSA:	Community-acquired MRSA
CC398:	Clonal Complex 398
CFU:	Colony Forming Units
CI:	Confidence Interval
CIb:	Centre for Infectious Disease Control
CLSI:	Clinical and Laboratory Standards Institute
COI:	Cost of illness
CTX-M:	Type of extended-spectrum beta-lactamase
CVI:	Central Veterinary Institute
DALY:	Disability Adjusted Life Years
DDD:	Defined Daily Dose
DHC:	Direct Healthcare Costs
DID:	Defined daily doses per 1000 Inhabitants per Day
DNHC:	Direct Non-Healthcare Costs
EARSS:	European Antimicrobial Resistance Surveillance System
EFSA:	European Food Safety Authority
EL&I:	Ministry of Economic Affairs, Agriculture and Innovation
EMEA:	European Medicines Agency
ESAC:	European Surveillance of Antimicrobial Consumption
ESBL:	Extended-spectrum beta-lactamase
ESCMID:	European Society for Clinical Microbiology and Infectious Diseases
EU:	European Union
EUCAST:	European Committee on Antimicrobial Susceptibility Testing
FAO:	Food and Agriculture Organization of the United Nations
FIDIN:	Association of manufacturers and importers of veterinary medicines in the Netherlands
GAP:	Good Agricultural Practices
GMO:	Genetically Modified Organism
GMP:	Good Manufacturing Practices
HACCP:	Hazard Analysis and Critical Control Points
HA-MRSA:	Hospital-acquired MRSA
HCW:	Healthcare workers
ICU:	Intensive Care Unit
IHC:	Indirect Healthcare Costs
IKB:	Integral chain control quality system
IMI:	Intra-mammary infection

INHC:	Indirect Non-Healthcare Costs
ISIS-AR:	Infectious diseases Surveillance Information System for Antibiotic Resistance
KNMvD:	The royal Dutch veterinary society
LA-MRSA:	Livestock-associated MRSA
LEI:	Agricultural Economics Institute
LNV:	Ministry of Agriculture, Nature and Food Quality (now EL&I)
LOS:	Length of stay
MARAN:	Report of VANTURES on Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands
MDR:	Multidrug-resistance
mecA:	Gene that renders *Staphylococcus aureus* resistant to all beta-lactam antimicrobials, among which methicillin
MIC:	Minimum Inhibitory Concentration
MLST:	Multilocus sequence typing
MRSA:	Methicillin-resistant *Staphylococcus aureus*
MSSA:	Methicillin-susceptible *Staphylococcus aureus*
NethMap:	Annual report of the SWAB and RIVM presenting data on human antibiotic use and resistance in the Netherlands
OIE:	World Organisation for Animal Health
OR:	Odds Ratio
OXA:	Type of extended-spectrum beta-lactamase
PFGE:	Pulsed Field Gel Electroforese
PVL:	Panton-Valentine leukocidin
RIVM:	National Institute for Public Health and the Environment
SCC*mec*:	Staphylococcal Cassette Chromosome mec
SFK:	Foundation for Pharmaceutical Statistics
SHV:	Type of extended-spectrum beta-lactamase
Spa:	Staphylococcus protein A gene
ST398:	(multilocus) Sequence Type 398
STEC:	Shiga toxin-producing *E. coli*
SWAB:	Dutch Foundation on Antibiotic Policy
TEM:	Type of extended-spectrum beta-lactamase
UK:	United Kingdom
USA:	United States of America
UTI:	Urinary tract infection
VANTURES:	The Veterinary Antibiotic Usage and Resistance Surveillance Working Group
VWA:	Food Safety Authority
VWS:	Ministry of Health, Welfare and Sport
WHO:	World Health Organization of the United Nations
WIP:	Dutch Workingparty on Infection Prevention
WT:	Wild type
WUR:	Wageningen University and Research centre
YLD:	Years Lost due to Disability
YLL:	Years of Life Lost

Classes of antimicrobials used in human and veterinary medicine.

Based on de Neeling et al. (1997) and EFSA (2008b).

Class	Mechanism of action	Use (H=human medicine, V= veterinary medicine)* and example antibiotics
MAIN CLASSES		
Beta-lactam antibiotics Subclasses: – Penicillins (J01C) – Penicillins – antistaphylococcal (J01C) – Cephalosporines, first-fourth generation (J01D) – Cephamycins (J01D) – Carbapenems (J01D)	Interference with cell wall synthesis	– H: amoxicillin, ampicillin, benzyl-penicillin V: amoxicillin, ampicillin, benzyl-penicillin – H: cloxacillin V: cloxacillin – H: cefalexin, cefuroxime, ceftazidime, cefpirome V: cefalexin, ceftiofur, cefquinome – H: cefoxitin V: - – H: ertapenem, imipenem, meropenem V: -
Aminoglycosides (J01G)	Inhibition of protein synthesis	H: amikacin, gentamicin, tobramycin V: gentamycin, neomycin
Tetracyclines (J01A)	Inhibition of protein synthesis	H: doxycycline, oxytetracycline V: chlortetracycline, doxycycline, oxytetracycline
Macrolides and ketolides (J01F)	Inhibition of protein synthesis	H: erythromycin, spiramycin, azithromycin, clarithromycin V: erythromycin, tylosin
Glycopeptides (J01XA)	Interference with cell wall synthesis	H: teicoplanin, vancomycin V: - (avoparcin formerly used as feed additive)
Folate pathway inhibitors (Sulphonamides and trimethoprim) (J01E)	Inhibition of metabolic pathways	H: sulfamethoxazole, trimethoprim V: trimethoprim-sulfamethoxazole, trimethoprim-sulfadoxin
(Fluoro)quinolones (J01M)	Interference with DNA/RNA synthesis	H: ciprofloxacin, norfloxacin, moxifloxacin V: danofloxacin, enrofloxacin
OTHERS		
Amphenicols (J01B)	Inhibition of protein synthesis	H: chloramphenicol V: florfenicol
Cyclic polypeptides (J01XX)	Interference with cell wall synthesis	H: bacitracin V: - (bacitracin formerly used as feed additive)
Ionophores (-)	Disruption of bacterial membrane structures	H: - V: - (monensin/salinomycin former feed additives)
Lincosamides (J01FF)	Inhibition of protein synthesis	H: clindamycin V: lincomycin, pirlimycin
Lipopeptides (J01XX)	Disruption of bacterial membrane structures, inhibition of protein synthesis, interference with DNA/RNA synthesis	H: daptomycin V: -
Nitrofurantoins (J01XE)	Inhibition of metabolic pathways	H: nitrofurantoin V: -
Nitroimidazoles (J01XD)	Interference with DNA/RNA synthesis	H: metronidazole V: -
Orthosomycins (-)	Inhibition of protein synthesis	H: - V: - (avilamycin formerly used as feed additive)

Class	Mechanism of action	Use (H=human medicine, V= veterinary medicine)* and example antibiotics
Oxazolidinones (J01XX)	Inhibition of protein synthesis	H: linezolid V: -
Pleuromutilins (-)	Inhibition of protein synthesis	H: - V: tiamulin, valnemulin
Polymixins (J01XB)	Disruption of bacterial membrane structures	H: colistin V: colistin
Quinoxalines (-)	Interference with DNA/RNA synthesis	H: - V: - (carbadox/olaquindox formerly used as feed additive)
Streptogramins (J01F)	Inhibition of protein synthesis	H: quinupristin/dalfopristin V: - (virginiamycin formerly used as feed
MISCELLANEOUS		
Fosfomycin (J01XX)	Interference with cell wall synthesis	H: fosfomycin V: -
Fusidic acid (J01XC)	Inhibition of protein synthesis	H: fusidic acid V: fusidic acid
Mupirocin (D06AX)	Interference with DNA/RNA synthesis, inhibition protein synthesis	H: mupirocin V: -
Rifampicin (J04AB)	Interference with DNA/RNA synthesis	H: rifampicin V: -

* - = not used

Erratum

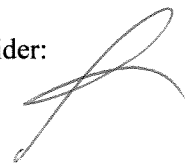
to report number 330334001/2010
"Risk profile on antimicrobial resistance transmissible from food animals to humans"

p.3, p.5, p.70 Table 14, p.90
On the above indicated pages of this report, it is mentioned that (quinolone-resistant) *Campylobacter* is mainly transmitted through consumption and preparation of broiler meat.
After printing of the report, it was noted that by using the word 'main(ly)' the attribution of (quinolone-resistant) *Campylobacter* to consumption and preparation of chicken meat was not properly presented. Contaminated chicken meat is an important source of human exposure in the Netherlands. It is estimated that at least 20% (with a maximum of 40%) of all cases of campylobacteriosis is directly or indirectly caused by contaminated chicken meat (RIVM report 250911009/2005). As mentioned on p. 90 of the present report, microbial subtyping has shown that approximately 50-80% of all human cases of *Campylobacter jejuni* can be attributed to the chicken reservoir (EFSA, 2009d).

p.68, p.76, p.91
We also want to update the figure of 13% genetic similarity between ESBL genes and plasmids in human clinical isolates and poultry isolates to 19% based on recent results (Leverstein-van Hall et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains, Clinical Microbiology and Infection, accepted article).

RIVM
Bilthoven
28 februari 2011

Ter akkoord

Paraaf Projectleider: 

Datum: 28 februari 2011

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