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Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections[1]

Scientific Opinion of the European Centre for Disease Prevention and Control; Scientific Opinion of the Panel on Biological Hazards; Opinion of the Committee for Medicinal Products for Veterinary Use; Scientific Opinion of the Scientific Committee on Emerging and Newly Identified Health Risks[2,3]

By alphabetical order:

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2 Advisory Forum (ECDC) members: http://www.ecdc.europa.eu/en/aboutus/organisation/af/Pages/AboutUs_Organisation_AdvisoryForum.aspx. BIOHAZ Panel Members (EFSA): Olivier Andreoletti, Herbert Budka, Sava Buncic, John D Collins, John Griffin, Tine Hald, Arie Havelaar, James Hope, Günter Klein, James McLauchlin, Winy Messens, Christine Muller-Graf, Christophe Nguyen-The, Birgit Noerrung, Luisa Peixe, Miguel Prieto Maradona, Antonia Ricci, John Sofos, John Threlfall, Ivar Vågsholm, Emmanuel Vanopdenbosch. EFSA-Q-2008-781 doi:10.2903/j.efsa.2009.1372 CVMP Members (EMEA): http://www.emea.europa.eu/htms/general/contacts/CVMP/CVMP_members.html and SAGAM/CVMP Members (EMEA): http://www.emea.europa.eu/htms/general/contacts/CVMP/CVMP_SAGAM.html. SCENIHR Members: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/scenihr_members_en.htm.

3 This joint report is based on major contributions of the following experts: ECDC: Anna-Pelagia Magiorakos, J. Todd Weber, Dominique L. Monnet. EFSA: J. Daniel Collins (Vice Chair), Seamus Fanning, Luisa Peixe, Chris Teale, John Threlfall (Rapporteur), in addition the BIOHAZ Panel wishes to thank EFSA's staff member Ernesto Liebana for the support provided to this scientific output. EMEA: Gérard. Moulin (Chair), Boudewijn Catry, Christian Friis, Kay Goodyear, Kari Grave, Cristina Muñoz, Jordi Torren, Karolina Törneke and John Threlfall (Rapporteur). SCENIHR: Jim Bridges, Herman Goossens, Philippe Hartemann, Jean-Yves Maillard, Jean-Marie Pagès. Correspondence: ECDC: info@ecdc.europa.eu, EFSA Panel's biohaz@efsa.europa.eu, EMEA: vet-guidelines@emea.europa.eu, SCENIHR: Sanco-Sc1-Secretariat@ec.europa.eu.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In June 2008, the Council adopted conclusions on antimicrobial resistance (AMR). The conclusions call upon the Commission and Member States to act in the area of healthcare associated infections, monitoring and control of AMR in humans and animals/food. As regards food- and animal-borne resistance, the Council calls upon the strengthening of surveillance on AMR and on the use of antimicrobials in the veterinary sector, the promotion of prudent use of antimicrobials, the promotion of mutual cooperation between all Directorates General and concerned agencies and cooperation with Member States (MS) the application of risk management strategies and the consideration of further control options when appropriate. AMR is indicated as a priority for the current and next presidencies.

Several scientific reports have been recently published by European and international scientific bodies, on the subject.

This mandate may be considered as part of preliminary risk management activities and its impact relative to other sources, on the impact of AMR in zoonotic infections in humans, as described in the "Principles and guidelines for the conduct of microbiological risk management" of the Codex alimentarius committee (CAC/GL 63 – 2007, available at: http://www.codexalimentarius.net/web/standard_list.jsp) and in the WHO/FAO Guide on Food Safety Risk Analysis (FAO Food and Nutrition Paper 87/2006, available at ftp://ftp.fao.org/docrep/fao/012/a0822e/a0822e.pdf). The Commission is in need of a scientific state of play in the area of AMR.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

For the purpose of this reply, an antimicrobial is defined as an active substance of synthetic or natural origin which destroys bacteria, suppresses their growth or their ability to reproduce in animals or humans, excluding antivirals and antiparasites.

The European Food Safety Authority (EFSA), The European Medicines Agency (EMEA), The European Centre for Disease Prevention and Control (ECDC) and The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) are requested to provide a common scientific report on the questions below based on the information currently available. The replies should be concise but references may be added supporting the statement.

ToR1. On the basis of the available scientific data on AMR in general, please provide a state of play and identify which additional data would be necessary to gain a proper understanding of the public and animal health problems linked to AMR, differentiated according to the source of resistance:

Use of antimicrobials in humans.

Use of antimicrobials in animals.

Others (if possible further differentiation might be considered e.g. may include antimicrobials used in plant protection, biocides, disinfectants, food preservatives, cosmetics, etc).

ToR2. Based on the existing data on AMR in zoonotic agents, which animal species/agent/antimicrobial combinations are considered of high concern and should be considered as a priority for the Commission?

For each of the combinations identified, indicate:

- To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent;
- Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?
- The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections, if not covered by term of reference (1) ("consequence estimate");

- To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species ("exposure estimate");
- To which extent a link between the use of antimicrobials in human medicine and the emerging/increase of AMR in humans exists
- To which extent a link between the use of the antimicrobial in animals and the emerging/increase of AMR in humans exists ("release estimate");
- To what extent alternative antimicrobials are available to prevent or treat animal diseases?

ToR3. Which are the areas where innovation and research should be encouraged in order to address existing problems caused by AMR?

In order to meet the deadline, parallel but coordinated discussions for different terms of reference or species/agent/antimicrobial combination might be considered.

APPROACH TAKEN TO ANSWER THE TERMS OF REFERENCE.

The ECDC (http://www.ecdc.europa.eu) is the EU agency that prevents and controls infectious disease in the EU. ECDC serves as information, knowledge and action centre to support and strengthen all EU institutions and countries in their work to detect, prevent and control infectious diseases. In order to achieve this mission, ECDC works in partnership with national health protection bodies across Europe to strengthen and develop continent-wide disease surveillance and early warning systems. By working with experts throughout Europe, ECDC pools Europe's health knowledge, so as to develop authoritative scientific opinions about the risks posed by current and emerging infectious diseases. AMR and healthcare-associated infections are the topics of a specific priority programme at ECDC which covers AMR issues in hospitalised patients as well as outpatients.

The EFSA (http://www.efsa.europa.eu) is the keystone of EU risk assessment regarding food and feed safety, and thereby including also antimicrobial resistance, as this has emerged in zoonotic bacteria. EFSA provides independent scientific advice and communication on existing and emerging risks. The Authority aims at using the best science available to carry out its tasks. Therefore EFSA mobilizes and coordinates scientific resources throughout the EU to provide high-quality and independent scientific advice and risk assessments. In practice, this takes place via scientific panels, working groups, task forces, grants and contracting scientific work as well as in other ways of networking with scientists. Requests for scientific assessments are received from the European Commission (Commission), the European Parliament (EP) and EU MS. EFSA also undertakes scientific work on its own initiative, so-called self-tasking. The BIOHAZ Panel and its supporting Scientific Unit, within the EFSA's Risk Assessment Directorate provides scientific advice on all questions on biological hazards relating to food safety and food-borne disease, including food-borne zoonoses and transmissible spongiform encephalopathies, microbiology, food hygiene and associated waste management.

The EMEA is a decentralised body of the European Union. Its main responsibility is the protection and promotion of public and animal health, through the evaluation and supervision of medicines for human and veterinary use. The mission of the EMEA (http://www.emea.europa.eu/) is to foster scientific excellence in the evaluation and supervision of medicines, for the benefit of public and animal health. The EMEA provides independent, science-based recommendations on the safety and efficacy of medicines, applying efficient and transparent evaluation procedures to help bring new medicines to the market by means of a single, EU-wide marketing authorisation granted by the Commission. This includes issues related to antimicrobial resistance derived from the use of medicines in humans as well as in animals. The Committee for Medicinal Products for Veterinary Use (CVMP) provides the scientific recommendations and opinions on veterinary medicines when these are related to antimicrobials, the Committee is supported by its Scientific Advisory Group on Antimicrobials (SAGAM). As indicated in the CVMP strategy on antimicrobials 2006-2010 (http://www.emea.europa.eu/pdfs/vet/swp/35329705.pdf), maintaining the efficacy of antimicrobials and minimising the development of AMR is one of the most important tasks in the field of veterinary medicine.

The SCENIHR, managed by the Unit Risk Assessment, Directorate-General Health and Consumers of the European Commission, is one of the three independent non-food Scientific Committees that provide the European Commission with the scientific advice it needs when preparing policy and proposals relating to

consumer safety, public health and the environment. These Committees also draw the Commission's attention to new or emerging problems which may pose an actual or potential threat. The SCENIHR deals with questions related to emerging or newly identified health and environmental risks and on broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. Examples of potential areas of activity include potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters and electronically controlled home environments), and methodologies for assessing new risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases.

The European Commission (DG SANCO) formally requested from ECDC, EFSA, EMEA and SCENIHR a close collaboration to address a mandate concerning antimicrobial resistance in zoonotic infections. The ECDC, the EFSA, with its Panel on Biological Hazards (BIOHAZ), the EMEA, with its Committee for Medicinal Products for Veterinary Use (CVMP), and the SCENIHR subsequently undertook this work. The three Agencies and the one Scientific Committee have worked in close collaboration, and in a coordinated fashion, for the preparation of common short Scientific Report. During the preparation of the paper the Agencies/Committees carefully took into account the terms of reference, and each Agency/Committee specifically addressed those areas within its own remit according to their own working practices. The development of the document was managed by an overarching working group with representatives from all bodies involved. The joint report has been formally adopted/endorsed by the four mentioned bodies.

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The EMEA wishes to thank the members of the *ad hoc* group organised with the purpose to answer the questions from the Commission; G. Moulin (CVMP Chair), J. Threlfall (Rapporteur), K. Törneke (SAGAM Chair), C. Friis, B. Catry, K. Goodyear, C. Muñoz and K. Grave as well as to the CVMP and SAGAM members.

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ASSESSMENT

1. Qualifying remarks.

- This document summarises the information provided in the background document (see Annex 1). It concentrates on conclusions in relation to the specific Terms of Reference, as provided by the Commission, including identification of knowledge gaps and suggestions regarding areas where innovation and research should be encouraged in order to address existing problems caused by AMR.

- For this document the term ‘antimicrobial’ has been used generically, to encompass antimicrobial agents, antibiotics and antibacterial agents. Strains of bacteria exhibiting resistance to antimicrobials are termed ‘antimicrobial-resistant’ and strains with resistance to more than one unrelated class of antimicrobial are termed ‘multidrug-resistant’ (MDR).

- Microbiological/epidemiological resistance, as opposed to clinical resistance, has been used wherever possible for this document. It should be realised that recommendations for the Minimal Inhibitory Concentrations (MICs) and epidemiological cut-off values for isolates deriving from animals and foods

were not harmonised in MS until 2008. Even after that date these are not necessarily used by all MS for AMR surveillance activities, particularly for isolates from cases of human infection. This has resulted in considerable difficulties in the comparison of human and animal data and in assessing trends in resistance in these species.

- The data presented refer for the most part to reports of studies made in MS, and to micro-organisms from human beings and food production animals from such countries. Nevertheless the supply of food commodities is a global undertaking, with food being imported into the EU from numerous countries where antimicrobial usage controls are not as strict as in the EU. Similarly a significant but non-quantified proportion of infections with antimicrobial-resistant strains in humans are acquired whilst travelling outside the EU and do not result from the consumption of contaminated food originating from within EU countries. Without detailed phenotypic and molecular subtyping the impact of such strains on the overall surveillance figures is difficult, if not impossible, to assess.

- Antimicrobial treatment, either for preventive or curative purposes, should never be considered as a first line approach in veterinary medicine. Management, hygiene, housing conditions, eradication and vaccination are key issues that need to be addressed first. In order to prevent dissemination of AMR a rational use of biocides might be considered. These issues apply to all organism/resistance combinations discussed in the document.

- Biosecurity measures and other strategies are of paramount importance to prevent and control disease and minimise the use of antimicrobials but such measures are not considered within the scope of this document.

- This document also concerns the occurrence of, and mechanisms of resistance to biocides in zoonotic bacteria.

2. ToR1. On the basis of the available scientific data on AMR in general, please provide a state of play and identify which additional data would be necessary to gain a proper understanding of the public and animal health problems linked to AMR, differentiated according to the source of resistance.

2.1. Introduction

AMR has increased worldwide in bacterial pathogens leading to treatment failures in human and animal infectious diseases. Resistance against antimicrobials by pathogenic bacteria is a major concern in the anti-infective therapy of both humans and animals. Bacteria are able to adapt rapidly to new environmental conditions and can acquire genes or undergo molecular changes with increasing exposure to antimicrobials in human and veterinary medicine, leading to resistance to these agents. Serious concerns about bacterial antimicrobial resistance from hospital-acquired, community-acquired and food-borne pathogens have been growing for a number of years and have been raised at both national and international levels. Both the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) consider AMR in zoonotic bacteria as a public health threat and recognize that resistance may be the consequence of the use of antimicrobials in food animals and may be transmitted to humans. This has been reflected in the establishment of a Codex *ad hoc* intergovernmental task force on AMR (http://www.codexalimentarius.net/web/index_en.jsp). Although the use of antimicrobials has been deemed to be the major factor in the development of bacterial resistance to these antimicrobials, the use of biocides (including disinfectants, antiseptics, preservatives, sterilants) may also make some contribution. Antimicrobials used for plant protection and metals (zinc, copper) with antimicrobial activity were considered to be outside the scope of this document.

Antimicrobials are invaluable compounds that provide society with numerous benefits when used appropriately. They play an important role in the treatment and control of bacteria in a variety of applications and are thus a precious resource that must be managed so as to be protected from loss of activity over time. Therefore, in order to preserve the role of such compounds in infection control and hygiene it is paramount to prevent the emergence of bacterial resistance and cross-resistance through their appropriate and prudent use.

2.2. General considerations

2.2.1. Antimicrobial agents

Antimicrobials are used extensively in both human and veterinary medicine world-wide for treatment and disease prevention. In food production animals, antimicrobials are also used for growth promotion; this practice has been forbidden in the EU since 2006. The use of antimicrobials in humans and animals is widely regarded as a major driving force in the emergence and spread of both AMR and antimicrobial-resistant bacteria.

2.2.2. Biocides

Biocides are widely used in many applications, including animal husbandry and in the food industry, e.g. animal feed preservatives, teat cleaning, for the disinfection of production plants and of food containers, and the control of microbial growth in food and drinks. In the EU feed preservatives are included in the category "technological additives" of feed additives under the Regulation (EC) 1831/2003 on additives for use in animal nutrition. Biocides are also extensively used in a wide range of applications in health care settings and in consumer products. Their use in food must be explicitly authorised at European level. In the laboratory, resistance to biocides has been linked to the appearance of resistance to antimicrobials, although such linkage has as yet not been conclusively identified in practice.

A clear connection between exposure to biocides and activation of the expression of different genes (structural and regulatory) involved in AMR has been recently demonstrated in important bacterial pathogens (*Escherichia coli* and *Salmonella*).

2.2.3. Bacteria

Resistance mechanisms in bacteria can be acquired or intrinsic. Bacterial species may exhibit a large variety of resistance mechanisms resulting in a final phenotype that may show resistance only to a single antimicrobial or to combinations of different antimicrobials. In addition, certain bacterial species show high levels of intrinsic resistance (*e.g.* impermeability of their membrane that strongly reduces antimicrobial penetration) and predisposition for the acquisition of additional resistance mechanisms. Such acquisition can take place through target mutations or the transfer of a mobile gene(s) from microbiota that share the same ecological niche.

2.2.4. Dissemination of antimicrobial-resistant bacteria and AMR genes

The dissemination of antimicrobial-resistant bacteria is a key contributor to the widespread emergence of problems in the treatment of infectious diseases. The dissemination and transmission of a specific resistance gene within the same bacterial species and its horizontal transmission from one bacterium to another (or to another bacterial species by means of a mobile genetic element) needs to be considered. Account also needs to be taken of the role of external factors which can promote the selection of bacteria exhibiting these resistance mechanisms, maintain the presence of resistance genes or favour the expression of specific complexes responsible for AMR.

2.3. Use of antimicrobials in humans

2.3.1. Humans - the target species

This report focuses on food-borne zoonotic bacteria commonly causing infections in humans and which are especially severe for immuno-compromised patients. The main reservoir of zoonotic bacteria is the gastrointestinal tract (GI tract) of healthy food animals, and most food-borne infections originate from faecal contamination of carcasses during slaughter, contamination of milk, or cross-contamination during subsequent processing.

The principal zoonotic bacteria causing gastrointestinal illness in humans in the EU are *Campylobacter* spp. (*Campylobacter*) and non-typhoidal *Salmonella* spp. (*Salmonella*), together accounting for the largest burden of

disease in the Europe and the North America, with an incidence that varies according to geographical region and causing increased morbidity and mortality. The main reservoir of these zoonotic bacteria is the gastrointestinal tract (GI tract) of healthy food animals, particularly poultry, cattle and pigs. Most food-borne infections originate from faecal contamination of carcases during slaughter or cross-contamination during subsequent processing.

The majority of salmonella and campylobacter infections result in mild, self-limited gastrointestinal illness and may not require antimicrobial treatment. Invasive disease, such as salmonella bacteraemia and meningitis and, rarely, campylobacter bacteraemia, can occur, with a higher risk in patients who are immuno-compromised. *Campylobacter* infections usually do not result in invasive disease as commonly as salmonella infections Hospitalization for invasive *Salmonella* has been estimated to be more than six times higher than for *Campylobacter*. The treatment of choice for salmonella infections is quinolones in adults and third-generation cephalosporins in children, and for campylobacter infections, macrolides or quinolones. Emerging resistance in *Salmonella* and *Campylobacter* is worrisome, as infections with antimicrobial-resistant strains cause inappropriate and delayed appropriate therapy that are associated with worse-patient outcomes, increased mortality and increased economic burden.

Other antimicrobial-resistant bacteria that may be considered zoonotic are vancomycin-resistant enterococci (VRE), non-Vero cytotoxin-producing *Escherichia coli*, and meticillin-resistant *Staphylococcus aureus* (MRSA), which can also be transmitted from animals through ingestion or direct contact. AMR is not considered important in infections caused by 'classic' food-borne *E. coli* pathogens such as Vero cytotoxin-producing *E. coli* (VTEC), and such organisms have therefore not been included in this list. *Escherichia coli* can also cause infections in humans ranging from urinary tract infections to bacteraemia and septic shock. Most of the *E. coli* isolates that can be traced to food are strains that cause gastrointestinal disease and have been attributed to transmission from meat contaminated during slaughter. Resistance to key therapeutic antimicrobials can seriously compromise treatment in extra-intestinal infections and urinary tract infections caused by strains of *E. coli* exhibiting such resistance. Infections caused by such antimicrobial-resistant strains are becoming increasingly common world-wide and are posing serious health problems for human medicine.

It should be noted that humans can become more susceptible to infection with antimicrobial-resistant zoonotic bacteria to which they are exposed. This can happen, when there has been prior use of antimicrobials, resulting in decrease in colonization resistance (dysregulation of intestinal microbiota) and an increased vulnerability to gastrointestinal illness with antimicrobial-resistant food-borne pathogens. This applies to all infections with all micro-organisms listed in this document.

2.3.2. Usage data

Details of overall human consumption of antimicrobials for systemic use, by antimicrobial class, in tonnes of active substance, in 29 European countries in 2007 or closest year available are provided in the background document (Annex 1). The figures provided are by antimicrobial class (ATC group), in tonnes of active substance (% total) sold, dispensed, or reimbursed by insurance systems, depending on the surveillance system, in 2007.

2.4. Use of antimicrobials in animals

2.4.1. Animals – the target species

Antimicrobials are used in veterinary practice in the treatment and control of infectious diseases such as pneumonia, enteritis, mastitis, peritonitis, and septicaemia as well as for local infections in a wide variety of food and companion animal species. Flock or herd administration of antimicrobials, which in most cases is given orally is considered one of the most important factors contributing to the selection of antimicrobial-resistant zoonotic bacteria. Companion animals are usually treated individually with antimicrobials.

2.4.2. Usage data

Ten European countries, of which eight are MS, were identified as having published data on the usage of veterinary antimicrobials. Data obtained through these programmes are published annually.

The usage of antimicrobials in animals in the various countries are reported as overall national sales, in terms of weight of active substance, while Denmark, the Netherlands and France also present usage data in various animal species. Overall national sales of veterinary antimicrobials in the different countries are presented in the background document. Such data were retrieved mainly from the latest reports from the various national surveillance programs.

2.5. Comparison of usage data in humans and animals

In the background document data on usage of antimicrobials in humans and animals were presented as overall national sales in weight of active substance. The interpretation of these data should be done with caution due to large differences between the doses applied among the various animals and humans and thus does not reflect the number of treatments received by either animals or humans. This limitation of weight of active ingredient as the unit of measurement also applies to the comparison of the usage of antimicrobials between human and animals as well as between countries, time periods etc. Also, the population of humans and animals treated varies considerably between the different countries and this further complicates the comparison.

2.6. Use of biocides

The use of biocides is not regularly monitored. The amounts of products applied or used remains largely unknown, despite their wide application and usage. Only general figures, such as the estimated EU-market value of €10-11 billion in 2006, with a continuing increase, are available. At present, in the absence of a mandatory monitoring system, no exact data on the amounts of substances used can be obtained.

Multidrug resistance (see below), together with increased levels of resistance to biocides has been demonstrated in laboratory-derived mutants of *Salmonella* Typhimurium, confirming the ability of biocides to select for such resistance. As yet, no naturally-occurring strains of *Salmonella* with biocide resistance linked to AMR have been reported.

3. ToR2. Based on the existing data on AMR in zoonotic agents, which animal species/agent/antimicrobial combinations are considered of high concern and should be considered as a priority for the Commission?

3.1. Combinations

The combinations below have been selected on the basis of current evidence of possible human health consequences.

3.2. Micro-organisms

The micro-organisms primarily addressed are *Salmonella* and *Campylobacter*. In the case of antimicrobial-resistant *E. coli* there are insufficient data about its role as a zoonotic bacterium. Information about the role of MRSA as a zoonotic bacterium is available from a report from the ECDC, EFSA and EMEA.

3.3. Antimicrobials

For the bacteria that this document focuses on, the following antimicrobial classes are considered of high concern: quinolones (including fluoroquinolones), cephalosporins (third- and fourth-generation); and macrolides. This is in accordance with the WHO categorization of Critically-Important Antimicrobials (CIAs) for Human Medicine.

3.4. Animal Species

In order to put most emphasis on the consequence for human health, the animal species component of the combinations has been omitted. Animal species are considered in more detail when answering detailed questions on each combination.

3.5. The Combinations

The following four combinations of micro-organism/antimicrobial resistance are regarded as of major concern and most relevance for public health:

- *Salmonella*/quinolone resistance
- *Campylobacter*/quinolone resistance
- *Salmonella*/cephalosporin resistance (third- and fourth-generation)
- *Campylobacter*/macrolide resistance

These combinations have been addressed individually in accordance with the specific questions under ToR 2.

4. Quinolone resistance in *Salmonella*

4.1. Mechanisms of resistance

Two fundamental types of quinolone resistance in zoonotic bacteria of importance to public health have been identified, namely chromosomally-mediated quinolone resistance and plasmid-mediated quinolone resistance (PMQR).

4.1.1. Chromosomal resistance

Chromosomal resistance to quinolones, arises spontaneously under antimicrobial pressure due to point mutations that result in: (i) amino acid substitutions within the topoisomerase II (DNA gyrase) and IV subunits *gyr*A, *gyr*B, *par*C or *par*E, (ii) decreased expression of outer membrane porins or alteration of LPS, or (iii) overexpression of multidrug efflux pumps. Mutations in the *gyr*A, *gyr*B, *par*C, or *par*E genes in regions that form the fluoroquinolone binding site (termed the Quinolone Resistance-Determining Region, QRDR) change the topoisomerase structure in a way that fluoroquinolones (FQs) are unable to bind to these target sites. Single mutations affect firstly only older quinolones such as nalidixic acid in their inhibitory action. The MIC for nalidixic acid is in the range of 64 – 128 mg/l, whereas the MICs for FQs are generally in the range of 0.25 – 1.0 mg/l. This level of resistance is generally regarded as 'epidemiological' (see above). Additional mutations are required to decrease the susceptibility to later (flumequine) and more recently-introduced FQs (ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, levofloxacin, marbofloxacin). These additional mutations result in the development of 'clinical resistance', with MICs of greater than 2 mg/l.

4.1.2. Plasmid-mediated quinolone resistance (PMQR)

Plasmid-mediated quinolone resistance (PMQR) is mediated by genes (*qnr*) encoding proteins that protect DNA gyrase from inhibition by ciprofloxacin. One such gene, *qnrA* confers resistance to nalidixic acid (MIC; 8-16 mg/l) and epidemiological resistance to FQs (ciprofloxacin MIC: 0.25 – 1.0 mg/l). The basal level of quinolone resistance provided by *qnr* genes is low and strains can appear susceptible to quinolones according to clinical laboratory standards institute (CLSI) guidelines. Their clinical importance lies in increasing the MIC of quinolone-resistant strains of *Salmonella* to levels that are clinically-relevant.

4.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent?

Over the five-year period 2000-2004, there has been an overall increase in cases of human infection in the EU by strains of *S.* Enteritidis exhibiting resistance to nalidixic acid and epidemiological resistance to ciprofloxacin, with the occurrence of resistance to both antimicrobials increasing from 10% to 26%. Over this period resistance remained constant at approximately 6% in *S.* Typhimurium. The highest incidence of resistance was seen in *S.* Virchow, with 68% of isolates resistant to nalidixic acid in 2002. Considerable variation in testing and reporting between countries was evident, and this variation was compounded by some countries reporting resistance at clinical rather than epidemiological/microbiological levels, and *vice-versa*. Isolates with PMQR have been reported in several countries, but such strains were mostly associated with travel to countries outside of the EU.

For isolates from foods, five countries provided data on the occurrence of resistance to nalidixic acid in *Salmonella* from pig meat in 2005 and six countries in 2006. As with isolates of *Salmonella* from humans there was considerable variation between different countries. In 2005 the incidence of resistance to nalidixic acid varied between 0% and 17% in pig isolates, and in 2006 from 0% to 10%. For broiler meat eight countries provided data for 2006. Overall, there was a high incidence of resistance to nalidixic acid, ranging from 13% to 90%. Resistance to ciprofloxacin was variable, with most countries not reporting resistance but with two countries reporting high levels (13% and 81% respectively).

Ciprofloxacin resistance was commonly found in isolates of *S.* Enteritidis from broiler meat and hens from countries in southern Europe but also from certain countries in northern Europe. With the exception of certain new MS, such resistance was relatively uncommon in isolates of *S.* Typhimurium from pork, pigs and cattle. With the exception of one northern European country, there was a high incidence of quinolone resistance in *Salmonella* from turkeys.

Comparison between the prevalence of resistance to quinolone antimicrobials in isolates of *Salmonella* from food animals, foods and cases of human infection is difficult because of differences in methodologies, and in interpretation of levels of resistance. Another reason for this difficulty is that there are differences in the number of isolates collected from food-producing animals (whether during routine surveillance or clinical evaluation), in countries undertaking such surveillance. Although results are indicative of developing trends, such as the increasing occurrence of resistance to quinolone antimicrobials in certain serovars and certain countries, to be meaningful it is vital that methodologies used for human and animal isolates are standardised and that systematic screening of representative strains (random sample of isolates with relevant sample size) from humans, food animals and food is undertaken by all MS. Another issue that creates difficulties in interpreting these data is the relative importance of antimicrobial-resistant *Salmonella* from imported food, which does not allow for a clear picture for domestic antimicrobial-resistant *Salmonella*.

4.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

Decreased susceptibility to quinolones usually results from chromosomal mutations in the QRDR, such mutations will give rise to decreased susceptibility to all members of this class of antimicrobials. In contrast to chromosomal mutations, the presence of PMQR genes does not always confer resistance to older quinolones – e.g., a *qnr* gene can result in decreased susceptibility to enrofloxacin although nalidixic acid is still active. A variety of serotypes and *qnr* genes (A1, B1, B2, B5, S1), have been frequently associated with genes conferring resistance to unrelated antimicrobials, including in particular extended-spectrum beta (ß)-lactamases (ESBLs) genes. In such serotypes *qnr* and ESBL genes are frequently present on the same plasmid backbone and may be co-transferred to suitable recipient strains. An association between cyclohexane resistance involving the over-expression of *Salmonella* efflux pump and an increased resistance to a number of antimicrobials including ciprofloxacin, triclosan, cetrimide has been demonstrated *in vitro*. Efflux pumps which contribute to quinolone resistance are also involved in the efflux of other antimicrobial classes e.g. polymyxins, phenicols.

4.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections

In the EU, *Salmonella* is the second most common human food-borne pathogen. From 2005 to 2006, EFSA Community Summary Reports show that resistance to nalidixic acid in *S.* Enteritidis increased from 13% to 15%, but resistance to ciprofloxacin remained stable at 0.4% - 0.6%. AMR in *Salmonella* has been associated with higher frequency and duration of hospitalisation, longer illness, a higher risk of invasive infection and a two-fold increase risk of death in the two years following infection. Infections with antimicrobial-resistant *S.* Typhimurium are associated with increased risk of invasive disease and death compared to susceptible infections and several studies have shown that patients infected with MDR *S.* Typhimurium definitive phage type (DT) 104 may have worse outcomes. Treatment failures, increased hospitalisation and higher risk of death have been reported for MDR *S.* Typhimurium DT104 exhibiting quinolone resistance.

4.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species – exposure estimate?

Foods have been implicated in several major national and international outbreaks of *S.* Typhimurium exhibiting epidemiological resistance to ciprofloxacin. Eggs contaminated with nalidixic acid-resistant *S.* Enteritidis have been linked to numerous outbreaks of salmonellosis in several European countries since 2000, although it was not possible to precisely ascertain how many infections have been associated with contaminated eggs. A series of studies in Denmark have demonstrated imported poultry and Danish eggs were important sources for quinolone-resistant *Salmonella,* and that travel was associated with the acquisition by consumers, of both MDR and quinolone-resistant *Salmonella*. Although infections with quinolone-resistant *Salmonella* associated with contact with domestic pets appear to be uncommon, concern has been expressed about the possibility of pet animals acting as reservoirs of antimicrobial-resistant *Salmonella*, including quinolone-resistant strains, particularly as antimicrobials, including fluoroquinolones, are used commonly in small animal veterinary practices.

4.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists?

There are no available data to evaluate a connection between the use of quinolone antimicrobials in humans and the widespread emergence of, or increase in resistance to this class of antimicrobial in *Salmonella*.

4.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists?

Several studies have shown that the use of FQs in food producing animals has resulted in the emergence of FQ-resistant isolates. Such strains have spread from food animals to humans.

In order to quantify to which extent a link between the use of antimicrobials in animals and emerging/increase of quinolone resistance in *Salmonella* from human exists, a quantitative risk assessment is needed. Elements provided in this report in terms of prevalence of bacteria and prevalence of resistance may help to focus on specific usages of medicines in different animal species, and highlight areas where further work is necessary to inform the debate on the link, if any, between the use of antimicrobials in animals and the emerging/increase of AMR in humans.

4.8. To what extent alternative antimicrobials are available to prevent or treat animal disease?

Quinolone-containing veterinary medicinal products may represent the only available treatment for certain indications in some animal species. Furthermore, for some serious indications alternative substances may either not be as efficient as quinolones, or their efficacy may have already been compromised due to the development of resistance. Older antimicrobials such as β-lactams (not associated with a β-lactamase inhibitor), sulphonamides, streptomycin and tetracyclines are possible alternatives, but resistance to these antimicrobials may be already present. Furthermore such antimicrobials are often subject to co-resistance. There are some antimicrobials

authorised for use in veterinary medicine for which resistance is rarely reported; for such antimicrobials the risks to human health linked to their use should be assessed.

5. Quinolone resistance in *Campylobacter*

5.1. Mechanisms of resistance

Quinolone resistance in *Campylobacter* is principally due to single mutations in *gyr*A and occasionally in topoisomerase IV (*par*C). The resultant MICs are in the range of 64-128 mg/l for nalidixic acid and 16-64 mg/l for ciprofloxacin. There is also evidence, albeit rarely, of resistance by efflux, with consequent cross-resistance to a range of therapeutic antimicrobials.

5.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent?

In 2005, 37% of *Campylobacter jejuni* and 48% of *C. coli* from cases of human infection in EU MS were resistant to ciprofloxacin. Comparatively, in 2006, 44% of *C. jejuni* and 58% of *C. coli* were resistant to ciprofloxacin and 31% of *C. jejuni* and 51% of *C. coli* were resistant to nalidixic acid. For isolates from animals, the occurrence of resistance to nalidixic acid among *C. jejuni* was particularly high in one MS, reaching almost 100%. These values were consistent from 2005 through 2007 for isolates from *Gallus gallus*. A similar trend was noted for ciprofloxacin. In contrast other countries reported a range of resistance from 0% to 3% for nalidixic acid, and where temporally comparable, a similar level for ciprofloxacin. A different profile was observed for the *C. coli* isolates, which showed an increased prevalence of resistance to quinolones ranging from 10% in pigs to complete resistance in isolates from *Gallus gallus*. In summary, available data would suggest that increasing quinolone resistance in isolates of *Campylobacter* from food animals may be reflected in clinical isolates from humans.

5.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

The Resistance-Nodulation-Division (RND) efflux pump CmeABC is known to contribute to intrinsic and acquired resistance to fluoroquinolones and macrolides in *C. jejuni* and *C. coli.* In addition to its role in mediating AMR, the CmeABC pump may also mediate resistance to bile salts, a key virulence feature.

5.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections

Direct data comparing human infections due to quinolone-resistant and quinolone-susceptible isolates of *Campylobacter* are not available. In the EU campylobacter infection has been the most commonly reported zoonotic illness from 2004-2007. Data for 2006 data show that 44% of *C. jejuni* and 58% of *C. coli* were resistant to ciprofloxacin, and 31% of *C. jejuni* and 51% of *C. coli* were resistant to nalidixic acid. Mortality in campylobacter infections is usually quite low, but tends to be higher in those patients with co-morbidities and when patients are infected with antimicrobial-resistant campylobacter strains. The health impact of infection with quinolone-resistant *Campylobacter* is of concern, because these infections are associated with longer duration of illness, and a greater risk of invasive disease or death. Adverse clinical events are increased 6-fold within 30 days of infection and 3-fold within 90 days, when patients were infected with quinolone-resistant compared to quinolone-susceptible strains. The evidence for a significant or added risk on public health of FQ resistance in *Campylobacter* is unclear. A meta-analysis of all such studies found no association.

5.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species – exposure estimate?

A number of case-control studies have specifically addressed risk factors for FQ-resistant *Campylobacter*. All of these studies identified foreign travel as a risk factor for acquisition of a FQ-resistant campylobacter infection, and this has also been highlighted in a study reported from Norway in 2005. In most of the studies it was not possible to conclusively say what the exposure food-stuff/route might have been although one study identified consumption of chicken and bottled water as risk factors for travel-related cases. Risk factors for non-travel related cases included use of a fluoroquinolone before the collection of the stool specimen, consumption of cold meat (pre-cooked), consumption of fresh poultry other than chicken and turkey, swimming (pool, ocean, lake or other places), consumption of chicken or turkey cooked at a commercial establishment and possession of non-prescribed antimicrobials. In relation to contact with domestic pets, over half of isolates recovered from cats and dogs in one MS were found to be resistant to nalidixic acid and ciprofloxacin as well as other antimicrobial classes, suggesting that companion animals should be considered as a further source of both MDR and ciprofloxacin-resistant *Campylobacter*.

5.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists?

There are no available data to evaluate a connection between the use of quinolone antimicrobials in humans and the emergence or increase in resistance to this class of antimicrobial in *Campylobacter*.

5.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists?

A temporal association between the emergence of quinolone resistance and its increase in isolates both from animals and humans following the introduction of this class of antimicrobial in animal production has been shown by several studies.

5.8. To what extent alternative antimicrobials are available to prevent or treat animal disease?

See 4.8 above.

6. Cephalosporin resistance in *Salmonella*.

6.1. Mechanisms of resistance

The main mechanism of resistance to cephalosporins is through production of β-lactamase enzymes which hydrolyse the β-lactam ring, thereby inactivating the cephalosporin (enzymatic barrier). The genes coding for these enzymes, of which there are a large number of different types, must be acquired by horizontal transmission from other bacteria since they are invariably absent from naturally-occurring *Salmonella* strains.

There are two broad types of β-lactamase enzyme which have been reported most frequently in *Salmonella* and which confer resistance to third-generation cephalosporins. These are:

(i) Extended-spectrum β-lactamases (ESBLs) (e.g. TEM and SHV variants and the CTX-M enzymes) these are class A enzymes in Ambler's molecular classification. They are inhibited by clavulanate and hydrolyse oxyimino-cephalosporins but not cephamycins.

(ii) AmpC β-lactamases which hydrolyse oxyimino-cephalosporins and cephamycins and are also resistant to clavulanate; they are class C enzymes in Ambler's molecular classification.

In addition to these types of β-lactamase, other types have also been reported, for example OXA enzymes, which are assigned to a different molecular class (class D) and the KPC enzymes (carbapenemases), which also confer resistance to cephalosporins.

Finally, mechanical barriers including impermeability of the bacterial cell wall can affect the level of resistance that is shown by the *Enterobacteriaceae* and may occur in conjunction with other resistance mechanisms. Additionally, as described for other *Enterobacteriaceae*, efflux pump activity may also contribute to ß-lactam resistance.

6.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent?

Meaningful comparison data on cephalosporin resistance in *Salmonella* from animals, food and humans is not feasible at present, due to the many differences that exist in assessing the data themselves. Differences in methods and discrepancies in data collection in AMR testing, in reporting procedures and possibly also in the lack of establishing epidemiological links between the three sources of bacteria, make a comparison not meaningful.

Comparisons made between the prevalence of resistance to cephalosporins in *Salmonella* from animals, food and humans without taking into account the different mechanisms that may confer such resistance may be misleading. Ideally, the same mechanism of resistance at least should be demonstrated in animal, food and human isolates of the same serotype to confirm that the isolates may be epidemiologically-linked. The available prevalence data do not always provide this level of detail and whilst broad comparisons may still be made, there is scope for results to be misleading unless further testing is performed.

A further issue is the antimicrobial used for detection of cephalosporin resistance in *Salmonella.* Enter-net and the EFSA Community Summary reports have reported resistance to cephalosporins in human cases of *Salmonella* based on the use of cefotaxime, a third-generation cephalosporin which is predictive of resistance to ceftriaxone. In contrast, ceftiofur, a different third-generation cephalosporin has been frequently used by many MS in their veterinary monitoring programmes prior to adoption of the EFSA guidelines. Ceftiofur has recently been found not to be a reliable antimicrobial for the detection of important mechanisms conferring resistance to third-generation cephalosporins. The conclusions that can be drawn from these EU ceftiofur resistance data in meat and food-producing animals are therefore limited and results from human isolates, and from animal and food isolates, may not be directly comparable.

Because the prevalence of resistance to third-generation cephalosporins in *Salmonella* from animals is currently low in all MS, it is not possible to provide information on trends in populations with confidence. The human, animal and food prevalences and reports of linkages between epidemiological groups show that transfer along the food chain can occur. The EU picture is also affected by global food imports and also by human travel-associated exposure to *Salmonella.*

6.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

Resistance to third- and fourth-generation cephalosporins in *Salmonella* is primarily caused by production of extended-spectrum β-lactamases (ESBLs) and/or AmpC enzymes. Both classes of enzymes confer resistance to extended-spectrum cephalosporins and to other β-lactam antimicrobials, with substrate specificity depending on the mechanism and sequential mutations involved. In particular, in different salmonella serovars ESBLs and/or AmpC enzymes have often been identified on plasmids. These plasmid-mediated resistances have frequently been found together with resistance determinants for e.g. aminoglycosides, chloramphenicol and florfenicol, sulphonamides, tetracyclines and/or trimethoprim, leading to efficient spread via co-selection. In addition, the down regulation of porins in some resistant isolates may also contribute to a decreased activity of antimicrobials that use the same entry pathway, such as FQs.

There are no data available to support a connection between the use of biocides and the emergence or increase in resistance to cephalosporins in *Salmonella.*

6.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections

Direct data comparing infections due to cephalosporin-resistant and cephalosporin-susceptible salmonella isolates are very limited. The most recent data on cephalosporin resistance in *Salmonella* from the EU are from 2006, when resistance of *S.* Typhimurium and *S.* Enteritidis to cefotaxime was 0.9% and 0.1%, respectively. Multidrug resistance was reported in 40% of *S.* Typhimurium and 0.7% of *S.* Enteritidis. There are only limited data on outcomes of human infections with cephalosporin-resistant *Salmonella,* although MDR salmonella infections have been shown to result in worse outcomes than infections with susceptible *Salmonella*

6.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species – exposure estimate?

The transmission of broad-spectrum cephalosporin resistance in *Salmonella* to humans either through the food chain or by direct contact between humans and animals has been conclusively demonstrated on only a few occasions. In the United States a ceftriaxone-resistant strain of *S.* Typhimurium which caused an infection in a child was linked to an outbreak in cattle on his father's farm. In EU MS the prevalence of resistance to third-generation cephalosporins in food-producing animals and meat appears to be low or very low, based on the available data. The data are not comprehensive and there are problems in making direct comparisons, as the data pre-date harmonised monitoring guidelines introduced in 2007 and are not harmonised or optimised for the detection of third-generation cephalosporin resistance. Nevertheless recent studies have reported the emergence and spread of a cephalosporin-resistant *S.* Virchow clone (carrying CTX-M-2) in poultry, poultry products and humans from 2000 in Belgium and France. The chronology of isolation suggested that the strain had been transmitted to humans by the food chain, probably by poultry meat. A similar spread was demonstrated for a clone of cephalosporin-resistant *S.* Infantis in poultry and humans in Belgium and France over the period 2001-2005.

In relation to human infection and domestic pets an association between handling pet treats containing dried beef and human infection with *S.* Newport expressing the AmpC β-lactamase CMY-2 has been demonstrated in Canada, illustrating the diversity of routes of transmission of antimicrobial-resistant *Salmonella* to humans.

6.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists?

There are no available data to evaluate a connection between the use of antimicrobials in humans and the emergence or increase of AMR, including cephalosporin resistance, in *Salmonella* in the EU.

6.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists?

Studies in cattle and swine have established a link between cephalosporin administration, including treatment frequency, and resistance selection in *E. coli*. *In vivo* transfer to, as well as the presence of, many of these ESBL genes in *Salmonella* has been demonstrated in several studies.

6.8. To what extent alternative antimicrobials are available to prevent or treat animal disease?

For almost all of the indications for which ceftiofur or cefquinome are authorised for systemic therapy in food producing animals, including salmonellosis, alternatives are available. An indication in which third- or fourth-generation cephalosporins could be the sole treatment are life-threatening invasive diseases such as septicaemia caused by *Enterobacteriaceae*.

7. Macrolide resistance in *Campylobacter*

7.1. Mechanisms of resistance

Modification of the macrolide ribosomal targets is the most common resistance mechanism encountered in *Campylobacter* spp. and this develops due to a mutation. Two nucleotides close to each other are target sites for modification. Mutation of A2075G results in a high-level erythromycin resistance (MIC > 128 mg/ml) in clinical strains of *C. jejuni* and *C. coli*. Since multiple copies of these genes exist, often a mosaic of resistance can be described, wherein not all targets are modified. A2074C or A2074T transversion mutations have been described in a clinical isolate of *C. jejuni* associated with an MIC > 128 mg/ml. Mutations affecting the ribosomal proteins L4 and L22 have also been identified. These were associated with both *C. jejuni* and *C. coli* that possessed a A2075G polymorphism in the 23S rRNA gene. A number of different mutations have been described in both ribosomal protein-encoding genes. In addition, the RND pump CmeABC is known to contribute to intrinsic and acquired resistance to fluoroquinolones and macrolides in *C. jejuni* and *C.coli*.

7.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent?

In the EU *Campylobacter* has been the most commonly reported gastrointestinal zoonotic illness from 2004 -2007. The EFSA Community Summary Report states that, in the EU in 2006, 23% of all *C. jejuni* and 10% of *C. coli* were resistant to erythromycin.

Campylobacter-associated enteritis is an important cause of morbidity across the globe and human exposure *via* retail chicken, including the types of *Campylobacter* involved, requires careful delineation. Various case-control studies have highlighted the risk associated with the consumption of contaminated chicken. Poultry can act as a reservoir to transmit drug resistant *Campylobacter* to humans. In a pan-European study involving five MS and using CLSI breakpoints and resistance levels based on EFSA epidemiological cut-off values, clinical resistance in isolates of *C. jejuni* cultured from chickens and cattle was absent and the occurrence of epidemiological resistance was low. Similar trends were observed for *C. coli.* In the UK, poultry meat was more frequently contaminated with *Campylobacter* (at a level of 53%) compared to *Salmonella* (7%), with chicken meat exhibiting the highest levels of contamination. In this study *C. coli* were more likely to exhibit AMR, including macrolide resistance, than *C. jejuni.* Comparing macrolide resistance between *C. jejuni* and *C. coli* isolates in south-eastern Italy, incidences of 3 % and 23 % erythromycin resistance was reported in *C. jejuni* and *C. coli* respectively from poultry and 4 % erythromycin resistance in *C. jejuni* from cattle. This study also highlighted differences in the propensity of *C. jejuni* and *C. coli* to become resistant to macrolides. In general, based on the observed trends *C. coli* would appear to be more resistant to macrolides compared to *C. jejuni*. In many studies most of the macrolide-resistant isolates of *C. coli* from food animals were of porcine origin, and this may reflect the chemotherapeutic choice made by veterinary practitioners in managing diseases other than campylobacteriosis in pigs.

Comparison between resistance in *Campylobacter* in the EU between animals, food and humans is difficult, as methods of testing and reporting by the MS are not standardised.

7.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

For macrolides, resistance selection by other antimicrobial compounds is common if *erm* genes - conferring resistance to macrolides, lincosamides and B-compounds of the streptogramins – are present - the so-called MLS_B phenotype. As yet this mechanism has not yet been described in *Campylobacter* species of major zoonotic importance. Mutations in the 23S rRNA target gene (domain V) often confer high-level macrolide resistance in *C. jejuni* and *C. coli* for the older macrolide groups such as erythromycin, azithromycin and tylosin, whereas ketolides (telithromycin, tulathromycin) may be less affected. Thus macrolide-resistant *Campylobacter* are resistant to macrolides used in human medicine, such as erythromycin, azithromycin and clarithromycin. In *Campylobacter*, the RND pump CmeABC is known to contribute to intrinsic and acquired resistance to macrolides, FQs, and β-lactams in *C. jejuni* and *C. coli*. In addition to its role in mediating resistance to antimicrobials, the CmeABC pump also mediates resistance to bile salts, a key virulence feature.

There are no data available to support a connection between the use of biocides and the emergence or increase of resistance to macrolides in *Campylobacter*.

7.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections

Direct data comparing infections due to macrolide-resistant and macrolide-susceptible isolates are limited. In 2006 2.3% of all *C. jejuni* and 10% of *C. coli* were resistant to erythromycin and multidrug resistance, defined as resistance to ≥4 antimicrobials, was reported in 8% of *C. jejuni* and 17% of *C. coli* isolates. Infections with macrolide-resistant *Campylobacter* are associated with an increased frequency of adverse events, including invasive illness and death compared to susceptible infections.

7.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species – exposure estimate?

Studies have demonstrated the occurrence of erythromycin-resistant *Campylobacter*, including *C. jejuni*, in retail raw meat samples and various foods, including chicken, raw milk, and the environment. A significant proportion of isolates were resistant to erythromycin, including 16% of isolates from chickens. From pets, similar proportions of erythromycin-resistant *C. jejuni* have been reported. Since onward transmission from domestic pets to humans is a recognised risk for contracting campylobacteriosis, this may be an important factor in the dissemination of macrolide-resistant strains of this pathogen.

7.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists?

There are no available data to evaluate a connection between the use of macrolide antimicrobials in humans and the emergence or increase in resistance to this class of antimicrobial in *Campylobacter*.

7.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists?

In a Canadian study which examined the resistance patterns of porcine *Campylobacter*, over 70% of isolates were resistant to macrolides. Risk analysis revealed a clear association between the (oral) administration of macrolides and the presence of resistance in faecal isolates.

There is controversy regarding the public health aspects of macrolide resistance in *Campylobacter*, with estimates based on a recent risk analysis not exceeding 1 out of 49,000 impaired human treatments in cases of infection with macrolide-resistant *C. coli* of porcine origin. The risk for suboptimal treatment of human cases due to macrolide- resistant *C. jejuni* infections from broiler and bovine sources was even lower.

7.8. To what extent alternative antimicrobials are available to prevent or treat animal disease?

Macrolides are primarily used to control gastrointestinal disorders in pigs and have a limited use for treatment of bovine mastitis. Furthermore, a number of new macrolides are used for treatment of respiratory infections. In most cases alternatives exist.

8. ToR3. Which are the areas where innovation and research should be encouraged in order to address existing problems caused by AMR?

- Improvement of surveillance activities and risk assessment
- Development and use of antimicrobials

- Development of new strategies to combat the diffusion of antimicrobial-resistant bacteria
- Assessment of possible contribution of other agents in the selection of antimicrobial-resistant micro-organisms

Further details on areas where innovation and research should be encouraged are provided in Chapter 11 of Annex 1.

CONCLUSIONS

General

AMR has increased worldwide in bacterial pathogens leading to treatment failures in infectious diseases in both humans and animals.

Harmonized epidemiological cut-off values for the detection and quantification of AMR in isolates of zoonotic bacteria from animals and foods did not come into place in MS until 2008 and even after that date have not necessarily used by all MS, particularly for isolates from cases of human infection. This has resulted in considerable difficulties in the comparison of human and animal data and in assessing trends in AMR in these species.

The supply of food commodities is a global undertaking, with food being imported into the EU from numerous countries where antimicrobial usage controls and regulations are different or not as strict as in the EU. Similarly a significant but as yet unquantified proportion of infections with antimicrobial-resistant strains in humans are acquired whilst travelling outside the EU and do not result from the consumption of contaminated food originating from within EU countries. Without detailed phenotypic and molecular subtyping the impact of such antimicrobial-resistant strains on the overall surveillance figures is difficult, if not impossible, to assess.

Micro-organisms

In general, AMR is not regarded as a serious problem in most of the *E. coli* strains that cause bacterial gastrointestinal disease in humans. In contrast resistance to key therapeutic antimicrobials can seriously compromise treatment in extra-intestinal infections and urinary tract infections caused by strains of *E. coli* exhibiting resistance to such antimicrobials. Infections caused by such antimicrobial-resistant *E. coli* strains are becoming increasingly common and further work is necessary to elucidate the source(s) of such strains.

Campylobacter and *Salmonella* together amount for the largest burden of gastrointestinal disease in humans in Europe. Should treatment be indicated, the antimicrobials of choice for salmonella infections are quinolones in adults and third-generation cephalosporins in children, and for campylobacter infections, macrolides or quinolones.

The main reservoir of antimicrobial-resistant strains of these zoonotic bacteria is the gastrointestinal tract of healthy food animals, particularly poultry, pigs, and cattle. Most food-borne infections with such antimicrobial-resistant strains originate from faecal contamination during slaughter or cross-contamination during subsequent processing.

AMR varies amongst both bacterial zoonotic pathogens and countries, thereby making the development of a single strategy to contain or reduce such resistance a difficult task.

Antimicrobial usage

Data on usage of antimicrobials in humans versus animals should be interpreted with caution due to large differences between the doses applied among the various animals and humans and thus do not reflect the number of treatments received by either animals or humans. These limitations of weight of active ingredient as unit of measurement also apply for comparison of the usage of antimicrobials between human and animals. Additionally variations between the populations of humans and animals treated as well as between countries and time periods further complicates comparisons.

The use of biocides is not regularly monitored, and the amounts of products applied or used remains largely unknown.

Combinations

The following four combinations of organism/antimicrobial resistance are regarded as of major concern and most relevance for public health in the EU:

- *Salmonella*/quinolone resistance
- *Campylobacter/*quinolone resistance
- *Salmonella*/cephalosporin resistance (third- and fourth-generation)
- *Campylobacter*/macrolide resistance

***Salmonella*/quinolone resistance**

General

Comparison between the prevalence of quinolone resistance in *Salmonella* from food animals, foods and cases of human infection is difficult because of differences in methodologies, in interpretation of levels of resistance. Another reason for this difficulty is that there are differences in the number of isolates collected from food-producing animals (whether during routine surveillance or clinical evaluation), in countries undertaking such surveillance.

Although results are indicative of developing trends, such as the increasing occurrence of resistance in certain serovars and certain countries, to be meaningful it is vital that methodologies used for human and animal isolates are standardised and that systematic screening of representative strains is undertaken by all MS.

The majority of *Salmonella* isolates with *qnr* genes from cases of human infection have been mostly associated with travel to countries outwith the EU. Of clinical concern is that the acquisition of plasmid-mediated quinolone resistance can raise the FQ MIC to clinical levels.

Specific

Chromosomal resistance to nalidixic acid and epidemiological/microbiological resistance to ciprofloxacin are virtually synonymous in isolates from all food animal species.

Plasmid-mediated quinolone resistance (*qnr*) is rare, but is increasing in incidence in isolates from both humans and animals.

The occurrence of clinical resistance to ciprofloxacin is low.

Epidemiological resistance to ciprofloxacin is common in *S.* Enteritidis from broiler meat and hens.

Resistance to ciprofloxacin and nalidixic acid is relatively uncommon in *S.* Typhimurium from pork, pigs and cattle.

There is a high incidence of resistance to quinolone antimicrobials in *Salmonella* from turkeys.

Multidrug resistance, together with increased levels of resistance to biocides has been demonstrated in laboratory-derived mutants of *S.* Typhimurium, confirming the ability of biocides to select for such resistance. As yet, no naturally-occurring strains of *Salmonella* with biocide resistance linked to antimicrobial resistance have been reported.

There are no available data to evaluate a connection between the use of quinolones in humans and the emergence or increase of quinolone resistance in zoonotic bacteria.

Some evidence is available on possible links between the use of quinolones in animal and emerging/increase of resistance in *Salmonella* from humans.

Rapid treatment for outbreaks of salmonellosis with an appropriate antimicrobial, in particular in equine clinics, is very important. Quinolone-containing veterinary medicinal products may represent the only available treatment for certain indications in some food-producing animal species.

Campylobacter/quinolone resistance

General

Susceptibility data for isolates from humans from different MS are not comparable for many reasons, e.g. lack of standardisation of susceptibility testing of human isolates between MS, inconsistent reporting by MS and the growing number of participating MS.

A temporal association between the emergence of quinolone resistance in *Campylobacter* and its increase in isolates both from animals and humans following the introduction of quinolones in animal production has been shown by several studies.

Specific

There are no available data to evaluate a connection between the use of quinolones in humans and the emergence or increase in resistance to this class of antimicrobial in *Campylobacter*.

Available data would suggest that the emergence of trends in quinolone resistance in animal isolates of *Campylobacter* may be reflected in clinical isolates.

Salmonella/cephalosporin resistance (third- and fourth-generation)

General

The prevalence of resistance to third- and fourth-generation cephalosporins in *Salmonella* from animals is currently low in all EU countries.

The same mechanism of resistance should be demonstrated in animal, food and human isolates of the same serotype to confirm epidemiological linkage. The available prevalence data do not always provide this level of detail.

In evaluating resistance testing, problems have arisen from the use of different breakpoints in the testing and interpretation of the results.

For testing for cephalosporin resistance in human isolates, resistance has been based on the use of cefotaxime. In contrast ceftiofur has been frequently used by many MS in their veterinary monitoring programmes. Ceftiofur has recently been found not to be a reliable antimicrobial for the detection of important mechanisms conferring resistance to third-generation cephalosporins. The conclusions that can be drawn are therefore limited and results from human isolates and from animal and food isolates may not be directly comparable.

Specific

There are no available data to evaluate a connection between the use of antimicrobials in humans and the emergence or increase of AMR, including cephalosporin resistance, in *Salmonella* in the EU.

The human, animal and food prevalences and reports of linkages between epidemiological groups show that transfer along the food chain can occur. Studies in cattle and swine have established a link between cephalosporin administration, including treatment frequency, and resistance selection in *E. coli*. *In vivo* transfer to, as well as the presence of, ESBL genes in *Salmonella* has been demonstrated in several studies.

In most cases the direct impact of infections with *Salmonella* strains resistant to cephalosporins on animal health is low.

A further increase of cephalosporin resistance can indirectly impact on animal health by increasing the prevalence of multidrug resistance, thereby severely reducing the number of effective alternatives for treatment.

Campylobacter/macrolide resistance

General

Macrolide-resistant *Campylobacter* are resistant to macrolides used in human medicine, such as erythromycin, azithromycin and clarithromycin.

Comparison between macrolide resistance in *Campylobacter* in the EU between animals, food and humans is difficult, as methods of testing and reporting by MS are not standardised.

Specific

Direct data comparing infections due to macrolide-resistant and macrolide-susceptible isolates in humans are not available.

Pets may act as a reservoir of macrolide-resistant *Campylobacter*.

There are no available data to evaluate a connection between the use of macrolide antimicrobials in humans and the emergence or increase in resistance to this class of antimicrobials in *Campylobacter*.

There is controversy regarding the public health implications of macrolide resistance in *Campylobacter*.

RECOMMENDATIONS

Details of recommendations that are relevant to zoonotic bacteria were collated from previous reports from the ECDC, EFSA, EMEA and SCENIHR, and are included in the background document (Annex 1).

GLOSSARY (DEFINITIONS)

Acquired resistance

A bacterial strain can acquire resistance by mutation, by the uptake of exogenous genes by horizontal transfer from other bacterial strains or by the activation/triggering of a genetic cascade, thereby inducing the expression of resistance mechanisms. Genes encoding enzymes that can modify the structure of an antimicrobial are commonly transferable. There are several genetic structures frequently acquired by horizontal gene transfer and which often function in concert. Large plasmids with many different genes can be transferred from bacterium to bacterium by conjugation. Such plasmids possess the necessary genes for self-conjugation. Small plasmids, which may carry between one and three resistance genes, but which do not carry conjugation genes, can also be transferred by mobilisation, often involving the presence of, or introduction of, a conjugative plasmid into carrier strains. Certain large plasmids which may have had their conjugation genes inactivated can also be transferred by mobilisation. Transposons can carry several resistance genes. They cannot replicate by themselves, but can move within the genome, e.g. from plasmid to plasmid or from chromosome to plasmid. Certain transposons conjugate intercellularly, being named conjugative transposons or integrative conjugative elements (ICEs). Integrons can also encode several resistance genes. They cannot move by themselves, but encode mechanisms both to capture new AMR genes contained within gene cassettes and to excise these from within and from the integron. Acquisition of resistance by mutation usually arises spontaneously due to point mutations that result, for instance, in changes in an antimicrobial target – e.g., chromosomal changes that result in resistance to quinolones and fluoroquinolones.

Antibiotic

A substance produced by, or derived (chemically produced) from a micro-organism that selectively destroys or inhibits the growth of other micro-organisms.

Antimicrobial

An active substance of synthetic or natural origin which destroys bacteria, suppresses their growth or their ability to reproduce in animals or humans, excluding antivirals and antiparasites.

Antimicrobial growth promoter

Antimicrobials used at low concentrations to stimulate an animal's growth, resulting in increased daily live weight gain and feed conversion efficiency.

Antimicrobial resistance

The ability of micro-organisms of certain species to survive or even to grow in the presence of a given concentration of an antimicrobial that is usually sufficient to inhibit or kill micro-organisms of the same species.

Biocide

An active chemical molecule that is present in a biocidal product and used to control the growth of or kill bacteria.

Biocidal products

Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, and which are intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.

Biofilms/planktonic bacteria

Biofilms are communal structures of microorganisms encased in an exopolymeric coat that form on both natural and abiotic surfaces. Planktonic bacteria correspond to cells growing in liquid/surface independent colonies without an exopolymeric coat or organized ultra-cellular structure.

Clinical resistance

The degree of resistance to a particular antimicrobial that results in therapeutic failure in treating an infection with that specific antimicrobial, even if the bacterium is exposed to maximum levels of this antimicrobial. The Minimum Inhibitory Concentration (MIC) of an antimicrobial for a bacterium isolated from clinical samples, in relation to assumed tissue concentrations in the infected patient, is used for guidance purposes. A bacterial isolate is categorized as clinically-resistant when the obtained MIC of the antimicrobial is associated with a high likelihood of therapeutic failure of treatment with that antimicrobial. Clinical breakpoints are intended for use in everyday clinical laboratory work to advice on therapy in the patient and may vary between countries and over time.

Commensal

An organism that derives benefit from living in close physical association with another organism or organisms. The latter organism(s) derive neither benefit nor harm from their relationship with the commensal organism.

Constitutive versus inducible resistance

Most resistance mechanisms are termed 'constitutive', because the resistance mechanism is always expressed. When the presence of an antimicrobial drug is required for the expression, such resistance is termed 'inducible'.

Co-resistance and co-selection

Genes conferring AMR are frequently contained in larger genetic elements such as integrons, transposons or plasmids, and as such may be 'linked' to other, unrelated resistance genes. In such cases, multiple resistance genes may be transferred in a single event. When two or more different resistance genes are physically linked, this is termed "co-resistance". Consequently, selection for one resistance attribute will also select for the other resistance gene(s), termed co-selection.

Cross-resistance

The tolerance to a usually toxic substance as a result of exposure to a similar acting substance. Antimicrobials are a diverse group of molecules, commonly ordered in classes with similar structure and mode of action. Within a class, the target in the bacterial cell and the mode of action of the antimicrobial is the same or similar in each case. Some mechanisms of resistance will confer resistance to most or all members of a class, i.e. cross-resistance.

Inherent (intrinsic) resistance

An inherent trait of certain bacterial species. For example, the target of the antimicrobial agent may be absent in that species, the cell wall may have poor permeability for certain types of molecules or the bacterial species may inherently produce enzymes that destroy the antimicrobial agent. These bacteria are clinically resistant, but should more accurately be referred to as "insensitive".

Microbiological/epidemiological resistance

The ability of a micro-organism to survive in the presence of antimicrobial concentrations (which may be lower than the clinical breakpoint) at which the micro-organism cannot normally survive. The MIC values used for this categorisation are termed "epidemiological cut-off values". The use of epidemiological cut-off values provides an appropriate level of sensitivity when measuring AMR development in bacteria of concern in both human and veterinary medicine.

Microbiota

The microbial flora that is associated with a particular tissue or organ in healthy animals/individuals.

Multidrug resistance

This term is used when a bacterial strain is resistant to more than one antimicrobial or antimicrobial class. There is no standard definition, which makes the term problematic and comparisons difficult. It is therefore important to define multidrug resistance in any document referring to 'multiple resistance'. Traditionally multidrug resistance is regarded as resistance to at least three different chemically-unrelated classes of antimicrobials, and is frequently transmissible. Strains exhibiting such resistance are termed 'multidrug-resistant' (MDR).

Pathogen

Any biological agent which can cause disease.

Zoonoses

Diseases or infections which are transmitted naturally between vertebrate animals and man.

ANNEX 1. BACKGROUND DOCUMENT ON ANTIMICROBIAL RESISTANCE (AMR) FOCUSED ON ZOONOTIC INFECTIONS BASED ON THE INFORMATION CURRENTLY AVAILABLE

TABLE OF CONTENTS

ASSESSMENT

1. GENERAL OVERVIEW OF THE MAIN HUMAN INFECTIONS DUE TO ZOONOTIC ANTIMICROBIAL-RESISTANT BACTERIA AND THOSE INFECTIONS INVOLVING AMR GENES.

Resistance to antimicrobials in bacteria causing infections in humans is a public health threat and can pose clinical problems. Additionally there is evidence of poorer outcomes in those patients with infections involving antimicrobial-resistant bacteria (Cosgrove, SE, 2006).

Food-borne diseases impose a significant burden on global human health. All-cause gastroenteritis is the second most common cause of morbidity and mortality in the world (Guerrant, RL *et al.*, 2001; Streit, JM *et al.*, 2006). Diarrhoeal disease is the third leading cause of Disability Adjusted Life Years (DALYs) globally (Murray, CJ and Lopez, AD, 1997). In the EU, over 350,000 zoonotic infections were reported in 2006. In the Netherlands, van den Brandhof *et al.* estimated that gastrointestinal disease was associated with a loss of 67,000 DALYs annually (van den Brandhof, WE *et al.*, 2004). In the United States, it is estimated that there are 76 million cases of foodborne illness each year, resulting in approximately 325,000 hospitalizations and 5,000 deaths (Mead, PS *et al.*, 1999).

1.1. Salmonella and *Campylobacter*

Campylobacter and *Salmonella* together account for the largest burden of disease in the Europe and the North America, with an incidence that varies according geographical region and causing increased morbidity and mortality (Mead, PS *et al.*, 1999). The majority of salmonella and campylobacter infections result in mild, self-limited illness and may not require treatment with antimicrobials. Diarrhoea, fever, and abdominal cramps are the dominant symptoms. Invasive disease, such as salmonella bacteraemia and meningitis and, rarely, campylobacter bacteraemia, can occur, with a higher risk in patients who are immuno-compromised (Pacanowski, J *et al.*, 2008). *Campylobacter* infections usually do not result in invasive disease as commonly as in salmonella infections (Helms, M *et al.*, 2005). Hospitalization for invasive non-typhoidal *Salmonella* has been estimated to be more than six times higher than for *Campylobacter* (Helms, M *et al.*, 2006).

Salmonella and campylobacter infections have been responsible for long-term sequelae. These include the Guillain-Barré syndrome, an acute inflammatory demyelinating disease causing flaccid paralysis, with an incidence of 1.3 cases per 100,000 cases of campylobacter infection (Nachamkin, I *et al.*, 1998) and, for both *Campylobacter* and salmonella infections, reactive arthritis (Doorduyn, Y *et al.*, 2008), inflammatory bowel disease (Gradel, KO *et al.*, 2009), and other autoimmune syndromes (Helms, M *et al.*, 2006).

The first-line treatment of choice for salmonella infections is quinolones in adults and third-generation cephalosporins in children, and for campylobacter infections, macrolides or quinolones (Guerrant, RL *et al.*, 2001). Infections with resistant strains cause delays in administration of appropriate therapy, and may result in worse outcomes (Helms, M *et al.*, 2005; Martin, LJ *et al.*, 2004; Molbak, K, 2005).

The main reservoir of these zoonotic bacteria is the gastrointestinal tract (GI tract) of healthy food animals, particularly poultry, cattle and pigs. Most food-borne infections originate from faecal contamination during slaughter or cross-contamination during subsequent processing.

1.2. Other relevant bacteria that may be considered zoonotic

Other antimicrobial-resistant bacteria that may be considered zoonotic are vancomycin-resistant enterococci (VRE), non-Vero cytotoxin-producing *Escherichia coli*, and meticillin-resistant *Staphylococcus aureus* (MRSA), which can also be transmitted from animals through ingestion or direct contact, colonising humans and sometimes causing infection.

Escherichia coli can cause infections in humans with symptoms ranging from gastroenteritis to bacteraemia and septic shock. Additionally *E. coli* may be responsible for extra-intestinal and urinary tract infections, which can

be asymptomatic or highly invasive. Most of the *E. coli* strains that have been traced to food are strains that cause gastrointestinal disease, and have been attributed to transmission from meat contaminated during slaughter. In general antimicrobial drug resistance is not considered important in infections caused by 'classic' food-borne *E. coli* pathogens such as Vero cytotoxin-producing *E. coli* (VTEC), which is why it has not been included in this assessment. In contrast resistance to key therapeutic antimicrobials can seriously compromise treatment in extra-intestinal and urinary tract infections caused by strains of *E. coli* exhibiting resistance to such antimicrobials. Infections caused by such antimicrobial-resistant non-Vero cytotoxin-producing *E. coli* strains are becoming increasingly common world-wide and are posing serious health problems for human medicine. At present there is controversy as to whether such strains can be regarded as zoonotic.

Enterococci are commensals in the animal and human intestine. Enterococci can become pathogenic and cause invasive disease, such as bacteraemia and endocarditis. There are reports of transmission of VRE to humans from animals, as well as horizontal transfer of resistance genes of isolates of animal origin to isolates in humans (Heuer, OE *et al.*, 2006; Lester, CH *et al.*, 2006). Additionally, transfer of the *vanA* gene from an *Enterococcus faecalis* isolate to MRSA can give rise to vancomycin-resistant *S. aureus* (VRSA) (Noble, WC *et al.*, 1992).

MRSA, which had initially emerged as a hospital-acquired pathogen (HA-MRSA), has since spread worldwide and represents a serious clinical problem in hospitals and other healthcare settings. In recent years, community-acquired MRSA (CA-MRSA) has emerged with no epidemiological connection with healthcare facilities and causing infection in humans. Very recently, livestock-associated MRSA (LA-MRSA) – mostly clonal complex 398 (CC398) – has been reported in some European countries. The primary reservoirs of MRSA CC398 in affected countries are pigs, veal calves and broiler chicken. MRSA CC398 has also been found in companion animals, horses, animal housing and surrounding environments of farms with colonized livestock. The relationship between antimicrobial usage and occurrence of livestock associated LA-MRSA in food production animals has been addressed by a recent EMEA reflection paper (EMEA/CVMP/SAGAM 68290/2009: "MRSA in companion and food producing animals in the European Union: Epidemiology and control options for human and animal health"). Information about MRSA as a zoonotic bacterium is also available at the EFSA[4] and EMEA[5] web pages.

1.3. Human health consequences of infections due to antimicrobial-resistant zoonotic pathogens

Resistance to antimicrobials in *Salmonella* and *Campylobacter* is of concern because if treatment is warranted, first-line antimicrobials may no longer be effective and treatment options are limited. More specifically, in antimicrobial-resistant *Salmonella* infections, quinolones, which are the drugs of choice in adults and cephalosporins, which are used in children, may not be active and appropriate empirical therapy may be delayed (Molbak, K, 2005). Inappropriate and delayed appropriate therapy are both associated with worse patient outcomes, increased mortality and increased economic burden (Barza, M, 2002; Kollef, MH, 2003; Lodise, Thomas Â P *et al.*, 2003). The evolution and dissemination of antimicrobial-resistant strains of food-borne bacterial pathogens in food animals and subsequently to humans, creates an increase in the "attributable fraction", the number of excess illnesses caused by antimicrobial-resistant zoonotic bacteria. This increases the risk of invasive infections, hospitalization and death associated with these bacteria. In an effort to mitigate the risk of AMR to human health arising from the use of antimicrobials in veterinary medicine, in 2007 WHO developed a list of antimicrobials according to how important they are in the treatment of human illness (Collignon, P *et al.*, 2009; WHO, 2007). Critically-important antimicrobials (CIAs) in this ranking include, among others, quinolones, macrolides, and third- and fourth-generation cephalosporins, which are the antimicrobials of choice in treating salmonella and campylobacter infections.

2. The use of antimicrobials in humans.

Data on antimicrobial use in humans are available from the European Surveillance of Antimicrobial Consumption (ESAC) network. Antimicrobials are grouped by class according to the Anatomical Therapeutic Chemical (ATC) classification and antimicrobial use is expressed as a number of Defined Daily Doses (DDD) per 1,000

4 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902590639.htm
5 http://www.emea.europa.eu/pdfs/vet/sagam/6829009en.pdf

inhabitants, and per day as recommended by the WHO Collaborating Centre for Drug Statistics Methodology (http://www.whocc.no/atcddd/).

The usage of antimicrobials in animals in the various countries are reported as overall national sales, in weight of active substance, while Denmark, the Netherlands and France also present usage data in various animal species. Overall national sales of veterinary antimicrobials in the different countries are presented in Table 2. Such data were retrieved mainly from the latest report from the various national surveillance programs

The latest data show a three-fold difference in outpatient consumption of antimicrobials for systemic use (ATC group J01) between the country with lowest use (the Netherlands: 11 DDD per 1,000 inhabitants and per day) and the highest use (Cyprus: 34 DDD per 1,000 inhabitants and per day). There were also large inter-country variations in hospital consumption of antimicrobials for systemic use (ATC group J01), from 1.2 to 3.5 DDD per 1,000 inhabitants and per day. More detailed information on antimicrobial consumption in humans can be obtained from the ESAC Yearbook 2007 (ESAC, 2009).

These data are difficult to compare with antimicrobial usage in animals. For comparability, data on the use of antimicrobials in humans in the EU in 2007 converted into tonnes of active compound were obtained from ESAC and are presented in Table 1.

Table 1. Overall human consumption of antimicrobials for systemic use, by antimicrobial class, in tonnes of active substance, 29 European countries, 2007 or closest year available. Source: European Surveillance of Antimicrobial Consumption (ESAC, 2009).

Country (Year)	Consumption[a] of antimicrobials for systemic use, by antimicrobial class (ATC group), in tonnes of active substance (% total)												
	Total (J01)[b]	Tetracyclines (J01A)	Amphenicols (J01B)	Penicillins with extended spectrum (J01CA)	β-lactamase sensitive penicillins (J01CE)	β-lactamase resistant penicillins (J01CF)	Combin. of penicillins with β-lactamase inh. (J01CR)	Cephalosporins and other β-lactams (J01D)	Sulphonamides and trimethoprim (J01E)	Macrolides, lincosamides and streptogramins (J01F)	Aminoglycosides (J01G)	Quinolones (J01M)	Other (J01CG, J01R, J01X)[g]
Austria (2007)[c]	37.8	0.5 (1)	0.0 (0)	4.5 (12)	6.1 (16)	<0.1 (<1)	11.3 (30)	4.0 (11)	0.5 (1)	7.1 (19)	<0.1 (<1)	3.3 (9)	0.4 (1)
Belgium (2007)[c]	78.9	2.0 (3)	0.1 (<1)	22.3 (28)	0.4 (1)	1.7 (2)	28.9 (37)	5.7 (7)	2.7 (3)	6.1 (8)	<0.1 (<1)	6.2 (8)	2.7 (3)
Bulgaria (2007)	62.6	1.5 (2)	0.5 (1)	23.4 (37)	4.3 (7)	0.0 (0)	6.1 (10)	11.9 (19)	5.4 (9)	3.6 (6)	0.3 (<1)	5.3 (9)	0.2 (<1)
Croatia (2007)	40.8	0.4 (1)	<0.1 (<1)	7.2 (18)	4.5 (11)	0.3 (1)	9.5 (23)	9.0 (22)	4.6 (11)	2.7 (7)	<0.1 (<1)	2.1 (5)	0.4 (1)
Cyprus (2007)	8.3	0.1 (1)	<0.1 (<1)	2.0 (25)	0.1 (1)	<0.1 (<1)	2.6 (32)	1.8 (22)	0.2 (2)	0.5 (6)	<0.1 (<1)	0.8 (10)	0.1 (1)
Czech Rep. (2007)[c]	53.2	1.0 (2)	0.0 (0)	7.6 (14)	15.2 (29)	<0.1 (<1)	10.8 (20)	2.9 (5)	6.3 (12)	5.8 (11)	<0.1 (<1)	3.4 (6)	0.3 (1)
Denmark (2007)	48.7	1.8 (4)	0.0 (0)	6.2 (13)	23.9 (49)	5.1 (10)	1.0 (2)	1.9 (4)	3.2 (7)	2.8 (6)	<0.1 (<1)	1.2 (2)	1.6 (3)
Estonia (2007)	6.2	0.2 (3)	0.0 (0)	1.9 (30)	0.4 (6)	0.1 (3)	0.9 (14)	1.0 (16)	0.5 (8)	0.7 (11)	<0.1 (<1)	0.5 (7)	0.1 (2)
Finland (2007)	39.0	3.3 (8)	0.0 (0)	6.2 (16)	6.8 (18)	0.4 (1)	3.6 (9)	13.3 (34)	1.2 (3)	1.9 (5)	<0.1 (<1)	1.9 (5)	0.5 (1)
France (2007)	645.3	12.4 (2)	0.1 (<1)	199.1 (31)	9.1 (1)	22.5 (4)	169.1 (26)	54.6 (9)	22.2 (3)	98.8 (15)	1.2 (<1)	40.2 (6)	16.0 (3)
Germany (2006)[c,d]	300.3	7.3 (2)	<0.1 (<1)	75.8 (25)	55.7 (19)	0.6 (<1)	10.6 (4)	41.6 (14)	41.3 (14)	44.1 (15)	0.6 (<1)	19.5 (6)	3.3 (1)
Greece (2006)	97.0	1.6 (2)	<0.1 (<1)	29.3 (30)	4.0 (4)	0.1 (<1)	18.2 (19)	29.0 (30)	0.0 (0)	5.1 (5)	0.7 (1)	6.6 (7)	2.3 (2)
Hungary (2007)	54.6	0.7 (1)	0.0 (0)	7.6 (14)	4.2 (8)	0.0 (0)	17.7 (32)	5.7 (10)	5.4 (10)	7.2 (13)	0.1 (<1)	5.3 (10)	0.8 (2)
Iceland (2006)[c]	2.0	0.1 (3)	0.0 (0)	0.4 (21)	0.5 (27)	0.2 (12)	0.3 (17)	<0.1 (1)	0.2 (10)	0.1 (6)	<0.1 (<1)	0.1 (3)	<0.1 (<1)
Ireland (2007)[c]	32.2	1.9 (6)	0.0 (0)	6.1 (19)	3.0 (9)	3.3 (10)	8.8 (28)	3.0 (9)	0.9 (3)	3.8 (12)	<0.1 (<1)	1.3 (4)	0.1 (<1)
Italy (2007)	599.0	2.2 (1)	2.3 (<1)	143.0 (24)	0.4 (<1)	0.7 (<1)	221.1 (37)	65.1 (11)	22.9 (4)	61.7 (10)	0.8 (<1)	47.9 (8)	31.0 (5)
Latvia (2007)	14.9	0.3 (2)	0.0 (0)	4.2 (28)	0.4 (3)	0.1 (1)	2.0 (13)	3.8 (25)	1.5 (10)	1.1 (8)	0.1 (1)	0.9 (6)	0.5 (3)
Lithuania (2006)	43.7	0.2 (1)	0.2 (<1)	1.5 (3)	31.3 (72)	2.0 (4)	1.4 (3)	2.9 (7)	<0.1 (<1)	0.9 (2)	1.8 (4)	0.8 (2)	0.7 (2)
Luxembourg (2007)	4.3	0.1 (2)	<0.1 (<1)	0.7 (17)	<0.1 (1)	0.1 (2)	1.6 (38)	0.7 (17)	0.1 (3)	0.4 (9)	<0.1 (<1)	0.4 (9)	0.1 (2)

Country (Year)	Consumption[a] of antimicrobials for systemic use, by antimicrobial class (ATC group), in tonnes of active substance (% total)												
	Total (J01)[b]	Tetracyclines (J01A)	Amphenicols (J01B)	Penicillins with extended spectrum (J01CA)	β-lactamase sensitive penicillins (J01CE)	β-lactamase resistant penicillins (J01CF)	Combin. of penicillins with β-lactamase inh. (J01CR)	Cephalosporins and other β-lactams (J01D)	Sulphonamides and trimethoprim (J01E)	Macrolides, lincosamides and streptogramins (J01F)	Aminoglycosides (J01G)	Quinolones (J01M)	Other (J01CG, J01R, J01X)[g]
Malta (2007)[e]	0.3	0.0 (<1)	<0.1 (<1)	<0.1 (2)	<0.1 (4)	<0.1 (2)	0.1 (39)	0.1 (31)	<0.1 (1)	<0.1 (7)	<0.1 (1)	<0.1 (5)	<0.1 (8)
Netherlands (2007)[c]	41.9	2.0 (5)	0.0 (0)	10.4 (25)	2.6 (6)	3.5 (8)	9.1 (22)	0.2 (1)	4.3 (10)	4.2 (10)	<0.1 (<1)	4.1 (10)	1.3 (3)
Norway (2007)	41.7	2.0 (5)	<0.1 (<1)	3.4 (8)	14.0 (34)	2.2 (5)	0.4 (1)	2.5 (6)	1.3 (3)	3.1 (8)	<0.1 (<1)	0.9 (2)	11.8 (28)
Poland (2005)[c]	217.9	4.1 (2)	0.0 (0)	93.9 (43)	7.9 (4)	0.1 (<1)	8.7 (4)	23.5 (11)	<0.1 (<1)	59.6 (27)	0.2 (<1)	14.3 (7)	5.6 (2)
Portugal (2007)[c]	58.0	0.3 (1)	<0.1 (<1)	7.8 (13)	0.2 (<1)	3.3 (6)	24.2 (42)	5.7 (10)	2.5 (4)	5.9 (10)	<0.1 (<1)	7.1 (12)	1.0 (2)
Slovakia (2007)[d]	69.6	0.3 (<1)	<0.1 (<1)	6.4 (9)	8.5 (12)	<0.1 (<1)	22.9 (33)	16.0 (23)	2.0 (3)	9.9 (14)	<0.1 (<1)	3.2 (5)	0.3 (1)
Slovenia (2007)	14.7	0.1 (<1)	0.0 (0)	2.6 (18)	3.3 (23)	0.3 (2)	3.7 (25)	1.0 (7)	1.7 (12)	1.0 (7)	<0.1 (<1)	0.9 (6)	0.1 (<1)
Spain (2007)[c,f]	288.6	1.2 (<1)	<0.1 (<1)	72.3 (25)	2.2 (1)	6.4 (2)	123.6 (43)	15.0 (5)	8.9 (3)	17.9 (6)	0.1 (<1)	30.5 (11)	10.4 (4)
Sweden (2007)	81.2	4.0 (5)	<0.1 (<1)	4.7 (6)	29.0 (36)	9.5 (12)	1.9 (2)	4.1 (5)	2.9 (3)	3.1 (4)	<0.1 (<1)	3.3 (4)	18.5 (23)
United Kingdom (2007)[c,d]	368.9	41.4 (11)	<0.1 (<1)	100.6 (27)	29.2 (8)	40.2 (11)	40.4 (11)	30.8 (8)	10.5 (3)	65.3 (18)	<0.1 (<1)	8.2 (2)	2.1 (1)

[a]Total consumption of antimicrobials for systemic use, i.e. outpatient and hospital sectors, unless otherwise indicated.
[b]Does not include polymyxins (J01XB). Total may exceed sum of consumption in each class as presented in this table due to rounding up to first decimal.
[c]Outpatient consumption of systemic antimicrobials only.
[d]Estimate based on average DDD for oral and parenteral administration since data by route of administration were not available.
[e]Hospital consumption of systemic antimicrobials only.
[f]Spain: reimbursement data, which do not include pharmacy dispensations without a medical prescription.
[g]Does not include polymyxins (J01XB).

3. The use of antimicrobials in animals.

3.1. General introduction

Antimicrobials are used in veterinary practice in the treatment and control of infectious disease such as pneumonia, enteritis, mastitis, peritonitis, and septicaemia as well as for local infections in a wide variety of food and companion animal species. Flock or herd administration of antimicrobials, in particular oral group treatments are amongst the most important factors contributing to the selection of antimicrobial-resistant bacteria. Companion animals are usually treated individually with antimicrobials.

In this report data on usage of antimicrobials in humans and animals have been presented as overall national sales in weight of active substance. These data should be interpreted with caution due to large differences between the doses applied among the various animals and humans and thus do not reflect the number of treatments received by either animals or humans. This limitations of weight of active ingredient as unit of measurement also applies for the comparison of the usage of antimicrobials between human and animals as well as between countries, time periods etc. Also, the population of humans and animals treated varies considerably between the different countries and this further complicates the comparison.

Antimicrobials are used by veterinary practitioners in the treatment and control of infectious disease in a wide variety of food and companion animal species. Treatments include single animal treatment or group treatment depending on the specific disease and animal production system.

The incidence of infectious diseases varies between animal species and consequently the choice of antimicrobials used in the treatment. In dairy cows mastitis, metritis, joint infections and foot rot are of major concern and are treated on an individual basis (Table 2). Pneumonia and enteritis are the most important infections in calves and are often treated by flock medications, when a certain percentage of animals are affected. This strategy is used to prevent clinical signs occurring in the remaining animals in the flock to reduce the spread of the infection. In weaning and slaughter pigs enteritis and pneumonia likewise are the most important infections and are treated by feed or water medication. In sows joint diseases and the mastistis-metritis agalactia syndrome frequently occur and are treated individually. In poultry outbreaks of enteritis and sinusitis occur occasionally.

Flock or herd administration of antimicrobials, in particular oral group treatment, is among the most important factors contributing to the selection of antimicrobial-resistant bacteria and is considered to be the major factor in contributing to a potential threat to human health.

In the period 1997-8, the EU withdraw the authorisation of antimicrobials used as feed additives (growth promoters) (avoparcin, virginiamycin, bacitracin, spiramycin and tylosin). The authorisation for use of four compounds as growth promoters (monensin, salinomycin, avilamycin and flavophospholipolin) was withdrawn in January 2006. No antimicrobial is now authorised for growth-promoting purposes in the EU.

To gain a proper understanding of the public health concern linked with antimicrobial resistance in zoonotic bacteria, it is important to consider not only the use of antimicrobials but also other factors linked to bacterial populations, AMR genes and the host, as well as the role of environment. These include:

- The characteristics of the bacteria under consideration, including their virulence and their capacity to spread, as well as the genetic basis of the AMR. Co-resistance should also be considered as in such cases use of several different antimicrobials might increase exposure to the same hazard.
- For food-borne hazards the rate of transfer is dependent on practices at slaughter and will differ between bacteria, animal species and production forms. It is clear that any measures to limit food contamination by bacteria would have an effect on minimising antimicrobial resistance problems for human health.
- Little is known about the extent to which commensal bacteria, or the AMR determinants carried by such organisms, transfer from animals to humans in the food chain.

- Both antimicrobial-resistant bacteria and AMR determinants are transferred across borders from country to country. AMR cannot be addressed only locally and a global approach is required. Measures applied only locally might have a limited impact.

3.2. Authorisation of antimicrobials in the EU

Before veterinary medicinal products (VMP), including antimicrobials, can be sold or supplied in the EU, premarketing evaluation by application of a harmonised procedure as established in the Commission Directive 2009/9/EC of 10 February, amending Directive 2001/82/EC is required. This directive provides detailed scientific and technical requirements regarding the testing of veterinary medicinal products. Market authorisation for a VMP is granted only after the product has undergone rigorous assessment on the criteria of safety, quality and efficacy. Safety includes the safety of the treated animals, the user of the product, the environment and the consumer of products from the treated animals. Applicants are required to address the microbiological properties of residues and the development of resistance (including resistance of relevance for clinical use in animals). Detailed guidance on how to address those points has been provided by the EMEA/CVMP and can be found at http://www.emea.europa.eu/htms/vet/vetguidelines/safety.htm.

Veterinary medicinal products, including antimicrobials, are registered in the EU according to the following procedures:

- National procedure - when the product is intended to be marketed in one single country of the EU.
- Decentralised /Mutual recognition procedure - when the product is intended to be marketed in several countries of the EU.
- Centralised procedure - the marketing authorisation will apply to all countries in the EU. Only innovative products i.e. new substances with new indications or with new innovative delivery methods or biotech products are eligible for the centralised route.

The EU requires by law that any food product (such as meat, milk or eggs) derived from animals treated with veterinary medicines must not contain any residue that might represent a hazard to the health of the consumer. Before a veterinary medicinal antimicrobial intended for food-producing animals can be authorised, the safety of its pharmacologically active substances and their residues must first be evaluated. The assessment of the safety of residues, including the possibility of a microbiological risk addressing both the development of AMR in bacteria of the human gut flora and disruption of the colonisation barrier, is carried out by the by the Committee for Medicinal Products for Veterinary Use (CVMP).

3.3. Which antimicrobials are used in animals on the basis of existing data

Most classes of antimicrobials used in animals are also frequently used in humans. In the EU some classes are currently solely used in humans (carbapenem and other penems, glycopeptides, synergistins and streptogramins, glycylcyclines, oxazolidinones, and lipopeptides).

Ten European countries, of which eight are MS, were identified as having published data on the usage of veterinary antimicrobials. Data obtained through these programs are published annually for the majority of these countries.

The usage of antimicrobials in the various MS are reported as overall national sales, in weight of active substance; Denmark, the Netherlands and France also present usage data per various animal species. In the present report overall national sales of veterinary antimicrobials in the different countries are presented (Table 2).

The sales figures presented in Table 2 should be interpreted with great care as the number/biomass of animals at risk for treatment with antimicrobials varies considerably between the different countries (Table 2). Furthermore, depending on factors such as potency, pharmacokinetic characteristics, formulation, MIC values and disease the dosages of various antimicrobials may vary considerably between substances. This has also to be taken into account when interpreting usage data. As an example taken from a French report, it is mentioned that: “Injectable

products with cephalosporins represent 1.4% of the tonnage of injectable antimicrobials sold; however, in terms of numbers of treatments, cephalosporins represent 10.7% of the injectable treatments sold".

As evident from Table 2 data are reported differently among the 10 countries; an example of this is the data for the use of cephalosporins, fluoroquinolones and macrolides. Usage of cephalosporins cannot be identified for two of the countries, as only total use of ß-lactams is given. It is also important to note that for those reporting sales figures for cephalosporins the amounts used of 3rd and 4th generation of cephalosporins cannot be identified. For those countries that report cephalosporin use in animals the percentage of such use varies between 1% to 7% of the total use (mean: 3%) (Table 2).

The percentage of use of macrolides in animals in the different MS reporting such use varies and is in the range of 5 % to 13 % (mean: 8%). As can be seen from Table 2 five countries report no use of lincosamides. Sales figures of lincosamides for these countries may have been included in the group of "as macrolides based on related spectrum and resistance mechanisms', and consequently the use of macrolides may be overestimated.

The amounts of fluoroquinolones used cannot be identified for three of the MS as only data on sales of quinolones are given. For those countries reporting such use the proportional use varies between 0.04% to 1% (mean: 0.64%) of the total use.

It should also be noted that, with the exception of one MS, the only usage of combination preparations presented in the various reports is for sulphonamides and trimethoprim. This implies that for other combination preparations the use is presented in relevant antimicrobial classes as sales of single substances or is not included in the data. The magnitude of use of combination preparations cannot therefore be identified.

Lack of a harmonized approach in terms of the reporting of data as well as incomplete information with respect to the use of CIAs, as identified by the WHO (WHO, 2007), partly confines the analysis of the data with respect to AMR. Another limitation of the data is that the usage of antimicrobials for local treatment (i.e. intramammary use) or for herd treatment (through feed and water) cannot be separated from the total use for the majority of countries.

A table listing the most common diseases in animals is included in Appendix A.

3.4. Comparisons of amounts of antimicrobials used.

A pragmatic approach to estimate of exposure intensity in animal and human populations has been used to express the amount, in weight of active ingredient, of antimicrobials sold for use in humans and animals in relation to the calculated biomass of humans and animals for the corresponding year.

Such a comparison has been done for French data for the period 1999 to 2005. In France the veterinary sector accounted for about 60% of the total amount of antimicrobials sold in France and the human sector for about 40% (data from 2005). The contributions of the two sectors differed with regard to the total animal and human biomasses estimated to have been treated annually. For example in 2005, the calculated ratio of mg of active ingredient sold per kg of live weight of potential users was 2.4 times higher in human medicine than in veterinary medicine.

Antimicrobial monitoring should be used to reveal trends of use of antimicrobials and to evaluate the results of management policies set up to reduce antimicrobial use.

3.5. Estimate of amounts of antimicrobials used

The inclusion criteria of veterinary antimicrobials in the surveillance programmes are inadequately described or are lacking for several of the countries. Therefore, it has not been possible to check whether the reported data include the same/all relevant ATCvet groups for all the countries.

Table 2. Overall national sales, in tons of active substance, of various classes of veterinary antimicrobials in 10 European countries. Data were retrieved from the latest report from the various national surveillance programs.

Drug classes (ATCvet groups*) / Year reported	Czech Republic[6] 2007	Den-mark[7] 2007	Finland[8] 2006	France[9] 2007	Germany[10] 2005	The Netherlands[11] 2007	Norway[12] 2007	Sweden[13] 2007	Switzerland[14] 2007	United Kingdom[15] 2007
Tetracyclines (QJ01A; QJ51A)	44.30	38.25	1.32	680.60	350.00	338.00	0.32	1.85	17.40	174.00
Amphenicols (QJ01B; QJ51B)	0.53	0.47	-	6.18	4.80	-	-	-	0.20	-
β-lactams (QJ01C; QJ51C;QJ01DB, DC, DD,DE; QJ51D)		36.32	8.86	121.72	199.20	64.00	2.86	9.46	12.90	66.00
β-lactams, penicillins (QJ01C;QJ51C)	12.96	35.66	7.86	112.47	-	-	2.86	8.51	12.40	60.00
Cephalosporins (QJ01DB, DC, DD ;DE; QJ51D)	0.42	0.66	1.00	9.25	-	-	N	0.95	0.50	6.00
Sulphonamides and trimethoprim (QJ01E)	-	14.65	2.95	257.81	97.5	101.00	1.64	2.87	29.40	73.00
Sulphonamides (QJ01EQ)	12.64	-	-	224.29	97.50	-	0.02	0.27	29.40	-
Combination of sulphonamides + trimethoprim (QJ01EW)	-	14.65	-	33.52	-	101.00	1.62	2.60	-	73.00
Macrolides and lincosamides (QJ01F;QJ51F)	-	16.54	0.62		64.70	58.00	0.02	1.52	3.70	33.00
Macrolides (QJ01FA; QJ51FA)	6.51	13.30	-	94.88	52.60	58.00	N	-	3.70	33.00
Lincosamides (QJ01FF; QJ51FF)	0.46	3.24	-		12.1	-	0.02	-	-	-
Aminoglycosides (QJ01G;QJ51G)**	0.89	8.13	0.23	74.82	36.3	12.00	0.17	0.718	3.80	20.00
Quinolones (QJ01M)		0.38	0.08	19.81	3.70	9.00	0.03	0.18	0.400	2.00
Fluoroquinolones (QJ01MA)	1.07	0.05	-	4.69	-	9.00	0.03	0.18	0.4	2.00
Other quinolones (QJ01MB)	-	0.33	-	15.12	-	-	-	-	-	-
Combination of antimicrobials (QJ01R;QJ51R)	-	-	-		-	-	1.265	-	-	-
Others***		7.77	0.07	93.04	28.20	8.00	0.20	0.51	4.20	14.00
Total use (tons)	79.36	123	14	1,349	784	590	6	17	72	382

- Not given *Substances or combination of substances belonging to QA07A (Intestinal antiinfectives), QG01A (Gynecological antiinfectives) and/or QJ01R and QJ51R (Combinations) may be included in the various relevant antimicrobial classes. **Finland has also included QJ01R (polymyxins) in this group. *** Included antimicrobial classes may vary from country to country.

6 Czech Republic, Bulletin of ISCVBM (Vestník ÚSKVBL)
7 Denmark, http://www.danmap.org/pdfFiles/Danmap_2007.pdf
8 Finland, http://www.evira.fi/uploads/WebShopFiles/1198141211941.pdf
9 France, http://www.afssa.fr/Documents/ANMV-Sy-Antibiotiques2007EN.pdf
10 Germany, http://www.bvl.bund.de/cln_027/DE/08__PresseInfothek/00__doks__downloads/Germap__2008,templateId=raw,property=publicationFile.pdf/Germap_2008.pdf
11 The Netherlands, http://www.cvi.wur.nl/NR/rdonlyres/A906A4C0-A458-423E-B932-28F222385988/52533/MARAN2005def.pdf
12 Norway, http://www.vetinst.no/nor/Forskning/Rapporter/Norm-Norm-Vet-rapporten/Norm-Norm-Vet-rapporten-2007
13 Sweden, http://www.sva.se/upload/pdf/Tj%C3%A4nster%20och%20produkter/Trycksaker/SVARM_2007%5B1%5D.pdf
14 Switzerland, http://www.swissmedic.ch/marktueberwachung/00147/00644/index.html?lang=en
15 United Kingdom, http://www.vmd.gov.uk/Publications/Antibiotic/salesanti07.pdf

4. The use of biocides.

This section refers to the opinion of SCENIHR on AMR effects of biocides (for detail see (SCENIHR, 2009)).

Biocides are extensively used in a wide range of applications in health care settings and in consumer products. The number of biocides in use is large. In the context of this mandate, biocides used for their surfactant properties, and for which the primary purpose is not their antimicrobial activity, as well as antimicrobial peptides (for instance, bacteriocins), will not be considered.

It can be noted that a biocide is usually used as part of a complex formulation and rarely on its own. Such formulation may contain more than one biocides, that when combined confer some increase in efficacy. In addition, components of the formulation might also potentiate the activity of a biocide (e.g. combination of quaternary ammonium compounds with EDTA, or biguanides with alcohols). In the literature, where the efficacy of a biocide is measured *in vitro*, the effect of the formulation is rarely considered. This is also the case in studies focusing on understanding mechanisms of bacterial resistance.

A clear connection between exposure to biocides and activation of the expression of different genes (structural and regulatory) involved in AMR has been recently demonstrated in important bacterial pathogens (*E. coli* and *Salmonella*) (Bailey, AM *et al.*, 2009).

4.1. Production, use and fate of biocides

In contrast to the surveillance on the use of antimicrobials used in human and animal health care, the use of biocides is not regularly monitored, and the amounts of products applied or used remains largely unknown (see SCENIHR opinion, Tables 1 and 2). Only general figures, such as the estimated EU-market value of €10-11 billion in 2006, with a continuing increase, are available. One important aspect of biocides is their use in healthcare settings; another is its use in consumer products. The following section focuses on the use of biocides in food production and animal husbandry.

4.1.1. Biocides in food production

Biocides are widely used in the food industry for the disinfection of production plants and of food containers, and as preservatives for control of microbial growth in food and drinks.

4.1.1.1. Biocides as disinfectants

Disinfection is regarded as a crucial step in achieving a defined, desired hygiene status in food production and processing areas, and in food processing plants. A variety of biocides are commonly used for the disinfection of equipment, containers, surfaces or pipework associated with the production, transport and storage of food or drink (including drinking water).

Disinfectants intended for use in the food-processing industry are regulated within the scope of Directive 98/8/EC on the placing of biocidal products on the market.

4.1.1.2. Biocides as food preservatives

Preservatives are substances which prolong the shelf-life of foodstuffs by protecting them against deterioration caused by micro-organisms. These compounds are considered food additives and are regulated by the Food Additives Directive 89/107/EEC[16]. Their use in food must be explicitly authorised at European level and they must undergo a safety evaluation before authorisation for using the preservative as intended.

16 European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners.

4.1.2. Biocides in animal husbandry

Proper cleaning and disinfection play a vital role in protecting food animals from endemic and zoonotic diseases, and thus indirectly protecting human health (for detailed accounts of all applications see chapter 3.3.4 of the SCENIHR report (SCENIHR, 2009)).

The use of biocides in animal husbandry follows the prerequisites set in the Biocides Directive 98/8/EC[17] that also invites MS to regulate the use of these agents. Consequently, some MS have published lists of authorised substances which are not harmonised. At present, in the absence of a mandatory monitoring system, no exact data on the amounts of substances used can be obtained. Although it appears that only a few disinfectant types are commonly used on a given farm, the same disinfectant brand may be used for extended periods of time.

In animal husbandry biocides are used as animal feed preservatives, with the aim of protecting feed against deterioration caused by micro-organisms. In the EU, feed preservatives are included in the category "technological additives" of feed additives under the Regulation (EC) 1831/2003[18] on additives for use in animal nutrition. Their use in feed must be explicitly authorised at European level. Before authorisation they must undergo a safety evaluation by EFSA.

Specific applications include biocides used as teat cleaners. The udders of animals used for milk production may be contaminated with faecal and other materials. Therefore, prior to milking, udders are cleaned with water that may contain biocides, alternatively, after the milking teat dips are applied to protect the milk duct and the entire udder from invading pathogens. Various chemicals are used for this purpose including chloroisocyanurates (which are organic chloramines), bronopol, quaternary ammonium compounds and iodine-based compounds.

Additionally, under the prerequisites of Directive 98/8/EC a range of disinfectants are permitted for decontamination in fish farming, for example for fish eggs, ponds and equipment. These include iodophores, metallic salts, halo-organic compounds, aldehydes, hydrogen peroxide, quaternary ammonium compounds and antimicrobial dyes.

4.1.3. Biocides in foods of animal origin

Regulation (EC) 853/2004 on specific hygiene rules for food of animal origin[19] constitutes the legal basis for the use of substances to remove surface bacterial contamination from products of animal origin, such as poultry carcasses. The use of these substances must be authorised by the European Commission (EC) after a safety assessment performed by the EFSA. Following a request from the EC, the EFSA has examined several substances used elsewhere in the world to decontaminate poultry carcasses. An assessment of the environmental impact of four substances (chlorine dioxide, acidified sodium chlorite, trisodium phosphate, peroxyacids) and their effect on AMR when used for the removal of microbial surface contamination of poultry carcasses was conducted by the SCENIHR, the Scientific Committee on Health and Environmental Risks (SCHER) (SCHER/SCENIHR, 2008), and by the EFSA BIOHAZ Panel (EFSA, 2008). At present none of these substances have been authorised for use in the EU.

4.1.4. General considerations on biocides

Current knowledge (including bacteriological, biochemical and genetic data) indicates that the use of certain active substances in biocidal products may contribute to the increased occurrence of antimicrobial-resistant bacteria, although only limited scientific evidence is available to correctly weigh the risks of AMR induced by resistance to biocides and some controversies remain. Some mechanisms of resistance are common to both biocides and antimicrobials (e.g. efflux pumps, permeability changes, biofilms). The selective pressure exerted by biocides may favour the expression and dissemination of certain mechanisms of resistance. The existence of horizontal gene transfer, particularly associated with mobile genetic elements, is the most likely mechanism for selecting and increasing AMR. The dissemination of these mobile genetic elements, their genetic capacity to

17 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31998L0008:EN:NOT
18 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32003R1831:EN:NOT
19 Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

contain several resistance genes and the presence of overlapping genetic cascades of regulation responding to selective pressures from chemicals on bacteria represent high risk factors.

It is important to establish the risks of selecting antimicrobial-resistant bacteria by biocides *in situ* to measure associated emerging health risks. Moreover, understanding the selection for, and dissemination of, biocide resistance in food-borne pathogens is important for combating health care-associated diseases.

5. Combinations (antimicrobial/micro-organism) considered to be of highest concern for human health.

5.1. General considerations

The combinations considered to be of highest concern have been selected on the basis of the current evidences of possible human health consequences. It is also important to stress that the answers to ToR2 and questions related to each combination are not intended to be an abbreviated risk assessment. This is a scoping exercise to define and describe the pertinent factors that may influence the risk posed by the hazard. It is particularly important to understand this in order to avoid any misunderstanding on the significance of the combinations selected. The primary criteria for the combinations selected were as follows:

5.2. Micro-organisms

Bacteria that are considered of high concern for human health, that are resistant to a particular antimicrobial class regarded as important for human health, and which can be transmitted through certain animal species to humans through the food chain.

Due to their relevance as zoonotic pathogens, and in light of the large number of infections per annum caused by these bacteria in the EU, *Salmonella* and *Campylobacter* are the focus of this document. Nevertheless the importance of addressing antimicrobial-resistant *E. coli*, MRSA and VRE is also recognised, although information in relation to their zoonotic involvement is not as comprehensive as for *Salmonella* and *Campylobacter*.

5.3. Antimicrobials

The antimicrobial classes considered of high importance are quinolones (including fluoroquinolones), cephalosporins (third- and fourth-generation only) and macrolides. This is in accordance with the 2007 WHO List of CIAs (WHO, 2007).

5.4. Animal Species

In order to put most emphasis on possible consequences for human health, it was agreed that, as a first approach, the animal species component of the combination would be omitted. Animal species are considered when answering the more detailed questions on each combination.

5.5. The Combinations

Taking into account the above criteria the following four combinations of organism/AMR were regarded as of major concern and most relevance for public health:

- *Salmonella*/quinolone resistance
- *Campylobacter*/quinolone resistance
- *Salmonella*/cephalosporin resistance (third- and fourth-generation)

- *Campylobacter*/macrolide resistance

These combinations have been addressed individually in accordance with the specific questions under ToR 2.

The combination of *Campylobacter*/cephalosporin resistance was excluded from the list, as cephalosporins are not used to treat campylobacteriosis in humans.

The combination *Salmonella*/macrolide resistance was also excluded from the list. In general macrolides are not used to treat salmonellosis in humans as *Salmonella* are intrinsically resistant to most macrolides. Nevertheless the macrolide antimicrobial azithromycin is increasingly being used to treat infections with strains of *Salmonella* Typhi which do not respond to treatment with fluoroquinolones, and also in some developing countries to treat invasive infections with MDR *Salmonella* other than *S.* Typhi.

6. Identification of additional data that would be necessary to gain a proper understanding of public health problem linked to AMR according to the use of antimicrobials in animals

Whilst the continued need to use antimicrobials in food animals for the purposes of health and welfare cannot be denied, measures to ensure that their use in food animals does not adversely impact human health are extremely important. It is therefore vital that the consequences of using antimicrobials at all stages in the food chain are clearly delineated and gaps in knowledge identified, in terms of both antimicrobial usage and the development of resistance to CIAs, as defined by WHO in key zoonotic bacterial pathogens causing infections in humans. These matters are addressed below in relation to the above combinations.

7. Quinolone resistance in *Salmonella*.

7.1. Brief description of the mechanisms of resistance.

Two fundamental mechanisms of antimicrobial resistance of importance to public health have been identified, namely chromosomal-mediated quinolone resistance and plasmid-mediated quinolone resistance (PMQR).

Chromosomal-mediated quinolone resistance.

In general, for *Enterobacteriaceae*, fluoroquinolones (FQs) are broad-spectrum antimicrobials highly effective for treatment of a variety of clinical and veterinary infections. Chromosomal resistance is due to inhibition of DNA replication and arises spontaneously under antimicrobial pressure due to point mutations that result in: (i) amino acid substitutions within the topoisomerase II (DNA gyrase) and IV subunits *gyr*A, *gyr*B, *par*C or *par*E, (ii) decreased expression of outer membrane porins or alteration of lipopolysaccharide (LPS), or (iii) over expression of multidrug efflux pumps. Mutations in the *gyr*A, *gyr*B, *par*C, or *par*E genes in regions that form the Quinolone Resistance-Determining Region (QRDR) change the topoisomerase structure in a way that fluoroquinolones are unable to bind to these target sites. Single mutations causing resistance, are found only in first-generation quinolones. Single mutations affect firstly only older generations such as nalidixic acid in their inhibitory action. The MIC for nalidixic acid is in the range of 64 – 128 mg/l, whereas and the MIC for FQs are generally in the range of 0.25–1.0 mg/l. This level of resistance is generally regarded as 'epidemiological'. Additional mutations are required to decrease the susceptibility to later (flumequine) and third-generation fluoroquinolones (ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, levofloxacin, marbofloxacin). Such additional mutations result in the development of 'clinical resistance', with MICs greater than 2 mg/l.

For *Salmonella*, the first reported example of high-level FQ-resistance was in serovar Typhimurium DT204 that emerged in animal feed and cattle in Belgium in the 1990s (Baucheron, S *et al.*, 2002). Studies have attributed FQ resistance in *S.* Typhimurium to an over-expressed AcrAB efflux pump and the involvement of efflux was confirmed through mutation (O'Regan, E *et al.* 2009, 2010). Although many studies focussed on FQ resistance, over-expression of efflux conferred a multidrug resistance phenotype. This up-regulation may arise from several mechanisms. Most of the genes encoding the broadly substrate-specific pumps are located on the bacterial chromosome whereas, antimicrobial-specific pumps may be encoded on transmissible plasmids (Quinn, T *et al.*,

2006; Yamane, K *et al.*, 2007), including one located within *Salmonella* Genomic Island 1 (SGI-1) in *S.* Typhimurium DT104. Additional mechanisms affecting the outer membrane permeability can also affect the susceptibility for quinolones, as demonstrated for *S.* Enteriditis. Bacterial efflux pumps extrude a broad range of structurally dissimilar compounds including antimicrobials such as fluoroquinolones, dyes and biocides

Plasmid-mediated quinolone resistance (PMQR)

Plasmid-mediated quinolone resistance (PMQR) was first identified in a clinical isolate of *Klebsiella pneumoniae* in 1988. PMRQ is mediated by genes (*qnr*) encoding proteins that protect DNA gyrase from inhibition by ciprofloxacin. One such gene, *qnr*A confers resistance to nalidixic acid (MIC; 8-16 mg/l) and epidemiological resistance to fluoroquinolones (ciprofloxacin MIC: 0.25-1.0 mg/l). The basal level of quinolone resistance provided by *qnr* genes is low and strains can appear susceptible to quinolones according to CLSI guidelines. Their clinical importance lies in increasing the MIC of quinolone-resistant strains to levels that are clinically-relevant.

During recent years horizontal dissemination of PMQR determinants (e.g. *qnr*A, *qnr*B, *qnr*S, *aac*(6')-Ib-cr, *qep*A) has contributed to the abundance of FQ resistance in *Enterobacteriaceae* (Cremet, L *et al.*, 2009; Hopkins, KL *et al.*, 2007; Yamane, K *et al.*, 2007). Within the EU PMRQ has now been reported in isolates of *Salmonella* from the UK (Hopkins, KL *et al.*, 2008; Murray, A *et al.*, 2008); France (Cattoir, V *et al.*, 2007), the Netherlands (MARAN, 2007; Veldman, K *et al.*, 2008), Portugal (Antunes, P *et al.*, 2009) and Denmark (Cavaco, LM *et al.*, 2007; DANMAP, 2007). The simultaneous presence of both chromosomal mutations in QRDR and PMQR has also been described (Cremet, L *et al.*, 2009).

7.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent.

7.2.1. Isolates from humans

Data for quinolone resistance in *Salmonella* from human infections can be found in reports from Enter-net and from EFSA (EFSA, 2006, 2007a, 2007b).

In an Enter-net study of 135591 isolates of cases of human infection in 10 European countries over the five year period 2000-04, the occurrence of resistance to ciprofloxacin (MIC: >1.0mg/l) remained constant at approximately 0.8% whereas resistance to nalidixic acid increased from 14% to 20% (Meakins et al, 2008). Although resistance to ciprofloxacin remained constant in most serotypes, for nalidixic acid considerable variation between serovars was observed. For example, in *S.* Enteritidis, the most commonly isolated serotype, resistance increased from 10% to 26% over the four-year period but remained constant at approximately 6 % in *S.* Typhimurium. The highest incidence of resistance to both ciprofloxacin and nalidixic acid was seen in *S.* Virchow, with 68% of isolates resistant to nalidixic acid in 2002 and approximately 4-5% of isolates exhibiting resistance to ciprofloxacin.

EFSA reported an increase in resistance to nalidixic acid in *S.* Enteritidis from 13% in 2005 to 15% in 2006, with considerable variation between countries; in contrast resistance to ciprofloxacin remained more or less constant at 0.4 -0.6% (Table 3). For *S.* Typhimurium resistance to nalidixic acid increased slightly from 6% to 8%, whereas resistance to ciprofloxacin remained constant at 0.6%-0.7%. Considerable variation between countries was again evident, and for ciprofloxacin resistance this variation was compounded by some countries reporting resistance at clinical rather than epidemiological/microbiological levels, and *vice versa*.

Table 3. Resistance to quinolones in human isolates of *Salmonella* Enteritidis and Typhimurium, European Union, 2005–06.

	2005		2006	
Serovar	Enteritidis	Typhimurium	Enteritidis	Typhimurium
Countries	15	14	15	14
Number studied	NS	NS	20148	5563
% Nal^R	13.4 (2- 52)	6.5 (0- 18)	14.8 (0 - 54)	7.8 (0-13)
% Cip^R	0.4 (0- 14.)	0.6 (0- 6)	0.6 (0 -15)	0.7 (0 - 4)

Range of % shown in parentheses; NS, not stated; Nal^R, nalidixic acid-resistant; Cip^R, ciprofloxacin-resistant

Source: (EFSA, 2006, 2007a, 2007b)

Foreign travel has been associated with importation of and human infection with quinolone-resistant *Salmonella*. In 2007 Denmark reported a 2% incidence of resistance to ciprofloxacin in domestically acquired infections with *S.* Typhimurium, compared to an 18% incidence in infections acquired abroad. The corresponding figures for nalidixic acid were 2% and 7% respectively. For *S.* Enteritidis the corresponding figures for domestically acquired and imported infections were 9% and 31% for both ciprofloxacin and nalidixic acid (DANMAP, 2007). In 2007 The Netherlands reported that in isolates of *S.* Enteritidis, which was the most frequent serotype related to travel, the overall resistance to ciprofloxacin and nalidixic acid was 13% and 12%, respectively, and for *S.* Typhimurium the corresponding incidences were 4% and 3%, respectively (MARAN, 2007). For the Netherlands, reports indicate that quinolone-resistant strains of *S.* Enteritidis were for the most part associated with foreign travel. In this respect it should be noted that the term 'foreign travel' encompasses travel to other MS as well as to countries outside the EU. An example of quinolone resistance in *Salmonella* specifically associated with travel to countries outwith the EU is that of infections with quinolone-resistant *S.* Kentucky in several EU countries, often related to travel specifically to countries in North Africa.

In contrast the majority of salmonella isolates with *qnr* genes from cases of human infection have been mostly associated with travel to countries outwith the EU. The increasing occurrence of plasmid-mediated quinolone resistance is of concern in that in strains already exhibiting decreased susceptibility to ciprofloxacin, the acquisition of PMQR can raise the MIC to clinical levels (see below).

7.2.2. Isolates from foods

For isolates from foods, five countries have provided data on the occurrence of resistance to nalidixic acid in *Salmonella* from pig meat in 2005 and six countries in 2006; details of serovars were not provided (EFSA, 2006, 2007a, 2007b). As with isolations of *Salmonella* from humans (see above), there was considerable variation between different countries. In 2005 the incidence of resistance to quinolones varied between 0% and 17% in pig isolations, and in 2006 from 0% to 10%. In 2005 1% of isolates reported by Denmark were resistant to ciprofloxacin, with similar levels in 2006. No ciprofloxacin-resistant or nalidixic acid-resistant isolates were recorded in 2007 (DANMAP, 2007). In 2006 1% of isolates from pig meat in Italy were reported as ciprofloxacin-resistant (EFSA, 2007b).

For broiler meat eight countries provided data for 2006 (EFSA, 2007b). Overall, there was a high incidence of resistance to nalidixic acid, ranging from 13% to 90%. Resistance to ciprofloxacin was variable, with most countries not reporting such resistance but with two MS reporting high levels (13% and 81% respectively).

In a series of UK studies quinolone-resistant strains of *S.* Typhimurium have been isolated from lamb and pork on retail sale (Little, CL *et al.*, 2008a), and quinolone-resistant *S.* Enteritidis from imported shell eggs (Little, CL *et al.*, 2007) and poultry meat (Little, CL *et al.*, 2008b). In the Netherlands a high incidence (>40%) of *S.* Paratyphi B variant Java (= *S.* Java) and other serovars with ciprofloxacin and nalidixic acid resistance in poultry meat was reported in 2007 (MARAN, 2007). In Denmark plasmid-mediated quinolone resistance has been

identified in three different serovars from imported turkey meat (see above). More recently PMQR (*qnrS*) has been identified in outbreaks of infection with *S.* Virchow in the UK. The source of infection was traced to imported cooked chicken. The causative strain was resistant to ampicillin, furazolidone and nalidixic acid, with concomitant epidemiological resistance to ciprofloxacin, and the presence of the *qnr* gene raised the MIC to ciprofloxacin to therapeutic levels (>1 mg/l) (Hopkins, KL *et al.*, 2007).

7.2.3. Isolates from food producing animals

Figures for *S.* Typhimurium isolates from cattle were provided from five countries in 2005 and from eight countries in 2006 indicated considerable variance in the occurrence of resistance to nalidixic acid (0%-26%). In contrast, the incidence of resistance to ciprofloxacin was low (0.2%). Similar figures were recorded for *S.* Typhimurium from pigs. In the case of *S.* Typhimurium isolates from turkeys, levels of up to 84 % resistance to nalidixic acid were reported in 2005 (EFSA, 2007a). For *S.* Enteritidis from *Gallus gallus* in 14 MS in 2006 the occurrence of resistance to nalidixic acid ranged from 0% to 95%, with an overall incidence of 28% (EFSA, 2007b). A 17% incidence of resistance to ciprofloxacin was reported from the Netherlands; the latter figures refer to epidemiological levels of resistance. In 2007 there was an incidence of 42% resistance to nalidixic acid/ciprofloxacin in *S.* Enteritidis from Dutch poultry, and of over 50% in *S.* Java from poultry (MARAN, 2007).

These findings have been confirmed by figures from a EFSA-funded study undertaken by the Danish Technical University (DTU) in 2009 (EFSA, 2009), investigating the occurrence of resistance to various antimicrobials in *Salmonella* from food production animals in MS from 2004-2007. For isolates from food-producing animals epidemiological resistance to ciprofloxacin was common in *S.* Enteritidis from broiler meat and hens, particularly in isolates from countries in southern Europe but also from certain countries in northern Europe. With the exception of certain new MS, such resistance was relatively uncommon in isolates of *S.* Typhimurium from pork, pigs and cattle. With the exception of one northern European country, there was a high incidence of quinolone resistance in *Salmonella* from turkeys.

Plasmid-mediated quinolone resistance has been identified in a single isolate of *S.* Infantis from poultry in Germany (Kehrenberg, C *et al.*, 2006), and single isolates of Bredeney (*qnrS1*) and Java from Dutch broilers (MARAN, 2007; Veldman, K *et al.*, 2008) and in Denmark from serovars Saintpaul (*qnrS1*), Newport (*qnrB51*) and Hadar (*qnrB5*) from imported turkey meat (DANMAP, 2007). The increasing occurrence of PMQR in *Salmonella* from food-producing animals is of concern in that in strains already exhibiting epidemiological resistance to ciprofloxacin, the acquisition of PMQR can raise the MIC to clinical levels.

Although the number of MS who have undertaken systematic screening of *Salmonella* isolates is relatively small, the above findings demonstrate that:

(i). Resistance to nalidixic acid and epidemiological/microbiological resistance to ciprofloxacin are virtually synonymous in isolates from all food animal species;

(ii). Plasmid-mediated resistance to quinolones is rare, but is increasing in incidence in isolates from both humans and animals;

(iii). The occurrence of clinical resistance to ciprofloxacin is very low;

(iv). Epidemiological resistance to ciprofloxacin is common in *S.* Enteritidis from broiler meat and hens, particularly in isolates from MS in southern Europe but also in certain MS in northern Europe;

(v). With the exception of certain new MS, resistance to ciprofloxacin and nalidixic acid is relatively uncommon in isolates of *S.* Typhimurium from pork, pigs and cattle; and

(vi). There is a high incidence of resistance to these antimicrobials in *Salmonella* from turkeys.

7.2.4. Comparison between those prevalences; their significance

Direct comparison of quinolone resistance data between the three categories - human / animal / food - is difficult. Certain MS, have used epidemiological cut-off levels (MIC: >0.125 mg/l) to define resistance to ciprofloxacin for *Salmonella* from food animals and foods (DANMAP, 2007; MARAN, 2007), whilst others have used epidemiological cut-off levels for isolates from animals and foods, but clinical levels (MIC: >1 mg/l) for defining resistance in *Salmonella* from cases of human infection. This is particularly the case with isolates from cases of human infection from 2000-04, as reported by Enter-net laboratories (Meakins, S *et al.*, 2008). Nevertheless certain trends are apparent, particularly the increasing occurrence in *S.* Enteritidis of nalidixic acid resistance coupled with epidemiological resistance to FQs in isolates from cases of human infection and in food isolates being mirrored by similar nalidixic acid-resistant strains from poultry meat (Antunes, P *et al.*, 2006; Little, CL *et al.*, 2008b; MARAN, 2007) and shell eggs (Little, CL *et al.*, 2007). There are also considerable 'between country' differences, with *Salmonella* from some MS exhibiting a high incidence of quinolone resistance, particularly in *S.* Enteritidis from poultry but also in certain new MS, in the occurrence of quinolone resistance in *S.* Typhimurium from pigs.

In conclusion, comparison between the prevalence of resistance to quinolone antimicrobials in isolates of *Salmonella* from food animals, foods and cases of human infection is difficult because of differences in methodologies, in interpretation of levels of resistance. Another reason for this difficulty is that there are differences in the number of isolates collected from food-producing animals (whether during routine surveillance or clinical evaluation), in countries undertaking such surveillance. Although results are indicative of developing trends, such as the increasing occurrence of resistance to quinolone antimicrobials in certain serovars and certain countries, to be meaningful it is vital that methodologies used for human and animal isolates are standardised and that systematic screening of representative strains (random sample of isolates with relevant sample size) from humans, food animals and food is undertaken by all MS. Another matter that creates difficulties in interpreting these data is the issue of introduction of strains of antimicrobial-resistant *Salmonella* from imported food or from human infections associated with foreign travel, which does not allow for a clear picture for domestic antimicrobial-resistant *Salmonella*.

7.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

Because the mechanism of PMQR can also involve active efflux systems, decreased susceptibility for other agents such as aminoglycosides may develop. In such strains *qnr* and ESBL genes are frequently present on the same plasmid backbone and may be co-transferred to suitable recipient strains.

As decreased susceptibility to FQs usually results from chromosomal mutations in the QRDR, such mutations will give rise to decreased susceptibility to all members of this class of antimicrobial (Lin, CC *et al.*, 2009). Principally all generations of quinolones will favour the selection of such chromosomal changes, for which the spread afterwards is clonal. The mechanism is stepwise and single mutations firstly affect only older compounds, such as nalidixic acid in their inhibitory action. Additional mutations are required to decrease the susceptibility to flumequine and newer FQs (ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, levofloxacin and marbofloxacin). When such cross-resistance for newer FQs arises, the older compounds are no longer active.

In contrast to the stepwise chromosomal mutations, the presence of PMQR does not always confer resistance to older quinolones e.g., a *qnr* gene can result in decreased susceptibility to enrofloxacin although nalidixic acid is still active. A variety of serotypes and *qnr* genes (A1, B1, B2, B5, S1), have been frequently associated with genes conferring resistance to unrelated antimicrobials including in particular ESBLs genes. In such strains *qnr* and ESBL genes are frequently present on the same plasmid backbone and may be co-transferred to suitable recipient strains. ESBL-positive *Salmonella* can carry structurally unrelated resistance genes affecting aminoglycosides, tetracyclines and sulphonamides. Many of these strains also harbour additional resistance mechanisms including PMQR (EMEA, 2009; Nollet, N *et al.*, 2006). Such different AMR genes gathered on a single mobile genetic element also are a major driver for co-selection. The resistance systems resulting from PMQR (e.g. *qnr*A, *qnr*B, *qnr*S, *aac*(6')-Ib-cr, *qep*A) often also show decreased susceptibility for other antimicrobials (e.g. aminoglycosides for *aac(6')-Ib-cr* gene). This is because the mechanism of resistance, which involves active efflux systems, is not only limited to quinolones.

Randall *et al.* (Randall, LP *et al.*, 2001) observed an association between cyclohexane resistance in *Salmonella* from poultry and increased levels of resistance to a number of antimicrobials, including ciprofloxacin. These isolates were also resistant to cetrimide and triclosan, possibly as a result of over expression of AcrAB. Similarly Karatzas *et al.* (Karatzas, KA et al., 2007) reported multidrug resistance in a low number of *S.* Typhimurium isolates following exposure to a QAC-aldehyde based disinfectant or an oxidising agent, The MIC to ciprofloxacin increased by 4-fold in these mutants. Laboratory-derived MDR mutants with an increased MIC to antimicrobials including ciprofloxacin (4-fold increase), were less fit than the parent strains (Randall, LP *et al.*, 2008). Although exposure to farm disinfectants might lead to the appearance of mutants with an increased MIC to quinolones in *Salmonella*, this might not be the case with other enterobacteria such as *E. coli* (Randall, LP *et al.*, 2005). As yet, no naturally-occurring strains of *Salmonella* with biocide resistance linked to AMR have been reported.

7.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections.

In the EU, *Salmonella* is the second most common human food-borne pathogen. From 2005 to 2006, EFSA Community Summary Reports show that resistance to nalidixic acid in *S.* Enteritidis increased from 13% to 15%, but resistance to ciprofloxacin remained stable at 0.4 %-0.6 %(EFSA, 2007a, 2007b).

Antimicrobial resistance in *Salmonella* is associated with higher frequency and duration of hospitalisation, longer illness, a higher risk of invasive infection and a 2-fold increase risk of death in the two years following infection (Desenclos, JC and Guillemot, D, 2004; Helms, M *et al.*, 2002). Infections with antimicrobial-resistant *S.* Typhimurium have been associated with increased risk of invasive disease and death compared to susceptible infections (Helms, M *et al.*, 2004; Molbak, K, 2005; Varma, JK *et al.*, 2005), and many studies have shown that patients infected with MDR *S.* Typhimurium DT104 may have worse outcomes (Devasia, RA *et al.*, 2005; Helms, M *et al.*, 2002; Martin, LJ *et al.*, 2004; Varma, JK *et al.*, 2005). Treatment failures, increased hospitalisation and higher risk of death have been reported for MDR *S.* Typhimurium DT104 exhibiting quinolone resistance (Molbak, K, 2005; Molbak, K *et al.*, 1999).

7.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species.

Food

Outbreak investigations

In 1998 an outbreak of MDR *S.* Typhimurium DT 104 with additional resistance to quinolones, in which 15 persons were affected, was traced through the food chain to pigs (Molbak, K *et al.*, 1999). In the same year an outbreak in the UK of MDR *S.* Typhimurium DT 104 exhibiting epidemiological resistance to ciprofloxacin, involving over 200 persons, and in which the vehicle of infection was milk, was traced to the farm of origin (Walker, RA *et al.*, 2000). In these two examples, the causative micro-organism was isolated from the food animal, from foods and from patients.

A further MDR strain (including resistance to ciprofloxacin) which has been associated with international food-borne outbreaks is *S.* Typhimurium DT 204b, with resistance to up to nine antimicrobials. In 2000 the strain was responsible for at least one major international outbreak involving 10 countries epidemiologically-linked to contaminated salad vegetables (Crook, PD *et al.*, 2003). Over 390 persons in five countries were infected with the epidemic strain (Crook, PD *et al.*, 2003). These infections accounted for approximately 1-2% of Typhimurium infections in Europe in 2000.

Attribution studies

Attribution of antimicrobial-resistant salmonella-related cases in Denmark has been investigated by Hald *et. al* (Hald, T *et al.*, 2008). In a study conducted in 2007 they considered the attribution of antimicrobial-resistant, MDR and quinolone-resistant *Salmonella* strains and concluded that: imported poultry and Danish eggs were important sources for quinolone-resistant *Salmonella*; pork (Danish and imported) and imported beef for MDR *Salmonella* infections; and Danish pork for antimicrobial-resistant salmonella infections. Additionally travel was

associated with the acquisition by consumers, of both MDR and quinolone-resistant *Salmonella* (Hald, T *et al.*, 2008).

Strains of *S.* Enteritidis resistant to nalidixic acid and with epidemiological resistance to ciprofloxacin have caused numerous infections in humans in the UK. Such strains have been linked to contaminated shell eggs used in the catering trade (O'Brien, S *et al.*, 2004), and particularly to eggs imported into the UK from Spain (Little, CL *et al.*, 2007). It was not possible to ascertain exactly how many infections with nalidixic acid-resistant *S.* Enteritidis have been associated with contaminated eggs, but from 2000 to 2004, in excess of 13,000 infections with nalidixic acid-resistant *S.* Enteritidis were recorded in 10 European countries (Meakins, S *et al.*, 2008).

Temporal studies

In the UK fluoroquinolones were licensed for veterinary use in 1993. Subsequent studies of the occurrence of resistance to quinolones in *S.* Typhimurium DT104 showed a temporal increase of quinolone-resistant isolates of MDR *S.* Typhimurium DT104 from humans, cattle, poultry and pigs (see Figure 1) (Threlfall, EJ *et al.*, 1999).

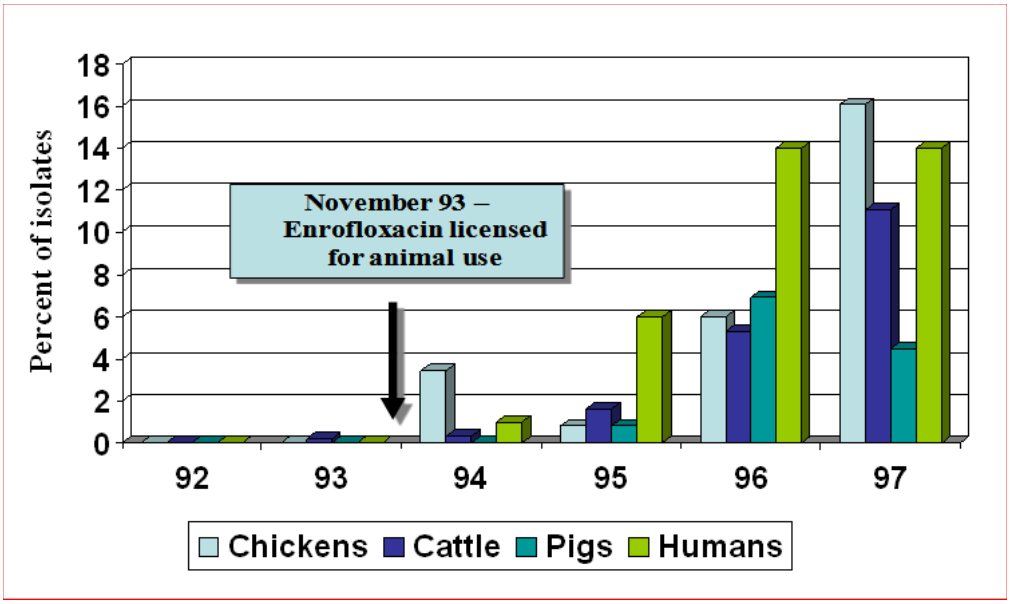

Figure 1 – Quinolone-resistant MDR *Salmonella* Typhimurium DT104,. United Kingdom, 1992-97

Pets

To date resistance transfer of *Salmonella* exhibiting resistance to quinolones from domestic pets to humans is rare in Member States. In the UK in 2009 tetracycline-resistant *S.* Typhimurium DT 191a associated with pet snakes have caused infections in humans. The source of the antimicrobial-resistant strain is thought to be imported frozen mice used as food for the reptiles (Anon, 2009). In the USA there have been reports of the transmission of strains of *S.* Typhimurium and *S.* Virchow exhibiting multiple drug resistance from pets to humans (CDC, 2001; Sato, Y *et al.*, 2000; Swanson, SJ *et al.*, 2007). In Australia, ornamental fish tanks have been identified as reservoirs for MDR *S.* Paratyphi variant Java (= *S.* Java) (Levings, RS *et al.*, 2006). Although not strictly from pets, in Canada MDR *S.* Newport associated with pet treats has caused infections in both humans and dogs (Pitout, JD *et al.*, 2003). Although infections with quinolone-resistant *Salmonella* associated with contact with domestic pets appear to be uncommon, concern has been expressed about the possibility of pet animals acting as reservoirs of antimicrobial-resistant *Salmonella*, including quinolone-resistant strains, particularly as antimicrobials, including FQs, are used commonly in small animal veterinary practices.

7.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists.

There are no available data to evaluate a connection between the use of antimicrobials in humans and the widespread emergence or increase in resistance to this class of antimicrobial in *Salmonella*.

7.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists.

Only limited information is available on this issue.

In order to quantify to which extent a link between the use of antimicrobials in animals and emerging/increase of quinolone resistance in *Salmonella* from human exists, a quantitative risk assessment is needed. Elements provided in this report in terms of prevalence of bacteria and prevalence of resistance may help to focus on specific usages of medicines in different animal species, and highlight areas where further work is necessary to inform the debate on the link, if any, between the use of antimicrobials in animals and the emerging/increase of AMR in humans.

In the UK, studies (Jones, YE *et al.*, 2002; Threlfall, EJ *et al.*, 1999) of the occurrence of resistance to quinolones in MDR *S.* Typhimurium DT 104 showed a temporal increase to this class of antimicrobial in isolates from humans, cattle, poultry and pigs from 1994 to 1997, following the licensing of FQs for veterinary use in 1993. More recent studies have shown that FQ resistance may be present at a high level (13 to 90% /2006 data) in *Salmonella* isolated in broiler meat. The level of FQ resistance in *Salmonella* from pigs was lower (0 to 10 % /2005 data), and very low in bovine isolates. In contrast there was a high incidence of resistance to quinolones in most MS in *Salmonella* from turkeys.

In a UK study of resistance in relation to antimicrobial usage in animals, recent changes in the incidence of quinolone resistance in isolates of *S.* Enteritidis and *S.* Typhimurium from humans did not correlate with the veterinary usage of quinolones. In the UK important factors in the increased incidence of quinolone resistance were foreign travel, and the consumption of imported foods contaminated with antimicrobial-resistant *Salmonella* (Threlfall, EJ *et al.*, 2006). These studies demonstrated that a number of factors contributed to changes in the incidence of resistance in predominant salmonellas in human infection in England and Wales from 2000 to 2004, and that antimicrobial usage in animals in a particular country was not always linked to changes in prevalence. In this respect there is very little definitive information on the issue of foreign travel and the importation of foods contaminated with antimicrobial-resistant salmonella bacteria from countries outside the EU.

The nature of quinolone resistance (QRDR and PMQR) does not allow a straightforward differentiation between an animals or human origin.

In order to quantify to which extent a link between the use of antimicrobials in animal and emerging/increase of quinolone resistance in *Salmonella* from human exists a quantitative risk assessment is needed. Elements provided in this report in terms of prevalence of bacteria and prevalence of resistance may help to focus on specific usages of medicines in different animal species, and highlight areas where further work is necessary to inform the debate on the link, if any, between the use of antimicrobials in animals and the emerging/increase of AMR in humans.

7.8. To what extent alternative antimicrobials are available to prevent or treat animal disease.

As rapid treatment for outbreaks of salmonellosis, in particular in veal calves and equine clinics with an appropriate antimicrobial is very important, quinolone-containing veterinary medicinal products may represent the only available treatment for certain indications in some food-producing animal species. Furthermore, for some serious indications alternative substances may either not be as efficient as quinolones or their efficacy may have already been compromised due to the development of resistance. Older antimicrobials such as β-lactams (not associated with a β-lactamase inhibitor), sulphonamides, streptomycin and tetracyclines are possible alternatives, but resistance to these antimicrobials may be already present. Furthermore such antimicrobials are

often subject to cross resistance. There are some antimicrobials authorised for use in veterinary medicine for which resistance is rarely reported; for such antimicrobials the risk to human health linked to their use should be taken into consideration when using those substances in animals. Wherever possible, biosecurity strategies should be implemented to minimise the use of all antimicrobials, including quinolones. Such strategies include vaccinations and adequate farming conditions and practices.

In some animal pathogens resistance to other authorised antimicrobial classes such as β-lactams, tetracyclines, trimethoprim and sulphonamides is widespread. Consequently, for some diseases antimicrobial therapy will be complicated if quinolones loose their activity. This is a risk for animal welfare and will result in economical losses. The best-documented example of this is *E. coli* septicaemia in poultry, because of the limited number of antimicrobials available for treatment of this animal species and the common presence of multidrug resistance (Bass, L *et al.*, 1999; Blanco, JE *et al.*, 1997). Other infections in which FQs are considered important for effective treatment are pneumonia in young cattle and sheep, severe mastitis caused by Gram-negative organisms and neonatal *E. coli* diarrhoea in piglets and calves (Prescott, JF *et al.*, 2000).

8. Quinolone resistance in *Campylobacter*.

8.1. Brief description of the mechanisms of resistance.

Quinolone resistance in *Campylobacter* is principally due to single mutations in *gyr*A and occasionally in topoisomerase IV (*par*C). The resultant MICs are in the range of 64-128 mg/l for nalidixic acid and 16-64 mg/l for ciprofloxacin (Engberg, J *et al.*, 2001). There is also evidence, albeit rarely, of resistance by efflux, with consequent cross-resistance to a range of therapeutic antimicrobials.

8.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent.

8.2.1. Prevalence data animals/food – humans

Human trends

In 2004, data on resistance to ciprofloxacin in *Campylobacter* were reported by six MS, with the lowest resistance in The Netherlands at 9% and the highest in Hungary with 50%. Resistance to nalidixic acid for 2004 was reported by four MS and was lowest at 10% in Norway and highest at 50% Hungary (EFSA, 2006).

In 2005, the EFSA-ECDC Community Zoonoses Report (EFSA, 2007a) stated that 37% of *C. jejuni* and 48% of *C. coli* were resistant to ciprofloxacin respectively. Comparatively, in 2006 44% of *C. jejuni* and 58% of *C. coli* were resistant to ciprofloxacin and 31% of *C. jejuni* and 51% of *C. coli* were resistant to nalidixic acid (EFSA, 2007b). Multidrug resistance (resistance ≥ 4 antimicrobials) in 2005 was found in 10% of all *C. jejuni* and in 14% of *C. coli*, whereas in 2006 8% of *C. jejuni* and 17% of *C. coli* were found to be MDR (EFSA, 2006, 2007b, 2007a). Data from 2007 are not yet available.

It is difficult to compare and draw meaningful conclusions from these data that are not directly comparable for many reasons, e.g. lack of standardisation of susceptibility testing of human isolates between MS, inconsistent reporting by MS and the growing number of participating MS.

A temporal association between the emergence of quinolone resistance and its increase in isolates both from animals and humans following the introduction of a fluoroquinolone antimicrobial in animal production has been shown by several studies (Endtz, HP *et al.*, 1991; Engberg, J *et al.*, 2001; Smith, KE *et al.*, 1999).

Animal trends

In 2008 EFSA recommended that MIC determinations for *Salmonella* and *Campylobacter* from food animals in the EU be altered from the use of clinical breakpoints to that of epidemiological cut-off values (EFSA, 2007c).

Data submitted by MS in regard to *C. jejuni* and *C. coli* covering the period 2004 through 2007 were assessed based on this quantitative measurement.

MS and non-MS submitted susceptibility data accordingly and the following lines provide a summary of the trends for quinolone resistance (including nalidixic acid and ciprofloxacin), for *C. jejuni* recovered from broiler meat, chicken (*Gallus gallus*) and cattle and for *C coli* recovered from the latter sources and pig meat. Data summaries reflect the analysis of ten or more isolates submitted by a country per sampling origin in a given year.

The occurrence of resistance to nalidixic acid among *C. jejuni* was notably high in one MS, reaching levels reflecting total resistance to this antimicrobial. These values were consistent from 2005 through 2007 for isolates cultured from *Gallus gallus*. A similar trend was noted for ciprofloxacin, a feature that reflects similar genetic mechanisms underpinning this particular phenotype. In contrast other countries reported a range of resistance from 0% to 3% for nalidixic acid, and where temporally comparable, a similar level for ciprofloxacin (EFSA, 2009).

A different profile was observed for *C. coli* isolates. In contrast to the above, *C. coli* appeared to show an increased prevalence of resistance to quinolone antibiotics, with resistance ranging from 10% (in isolates cultured from pigs being recorded in 2007) to 100% resistance in isolates from *Gallus gallus*. These trends appeared to be of greater concern when associated with *Gallus gallus* and pigs than when compared to isolates from broiler meat or from cattle.

In comparing *C. jejuni* isolates from *Gallus gallus* and broiler meat in 2007, three MS submitted data that could facilitate a direct comparison. The only significant difference detected in regard to quinolones showed that between isolates, sources levels were comparable and between countries, one non-MS appeared to have higher levels of resistance in both sources. In the latter case isolates from broiler meat were found to be more resistant. A similar observation in respect of *C. coli* could be made for one of the non-MS.

In an earlier independent study, AMR profiles of strains recovered from retail food and humans following a 3-year surveillance programme in one MS were investigated. The susceptibility patterns to a panel of eight antimicrobials were determined by disc diffusion (McGill, K *et al.*, 2006). Resistance to erythromycin, ciprofloxacin and tetracycline was low among isolates from food and similar to that of temporally-matched clinical isolates.

8.2.2. Comparison between those prevalences; their significance

In summary, the above data would suggest that the emergence of trends in quinolone resistance in animal isolates of *Campylobacter* may be reflected in clinical isolates from humans.

8.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

In *Campylobacter*, the RND pump CmeABC is known to contribute to intrinsic and acquired resistance to fluoroquinolones and macrolides in *C. jejuni* and *C. coli* (Corcoran, D *et al.*, 2005; Quinn, T *et al.*, 2007). In addition to its role in mediating resistance to antimicrobials, the CmeABC pump also mediated resistance to bile salts, a key virulence feature.

Co-selection can result from distinct resistance mechanisms simultaneously present both in mobile genetic elements and in strains. A common co-resistance often encountered in quinolone-resistant *Campylobacter* is to tetracyclines

8.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections.

Direct data comparing human infections due to quinolone-resistant and quinolone-susceptible isolates of *Campylobacter* are not available.

Data for 2006 data show that, 44% of *C. jejuni* and 58% of *C. coli* were resistant to ciprofloxacin, and 31% of *C. jejuni* and 51% of *C. coli* were resistant to nalidixic acid. Mortality in campylobacter infections is usually quite low, but tends to be higher in those patients with co-morbidities and when patients are infected with AR *Campylobacter* strains. The health impact of infection with quinolone-resistant *Campylobacter* is concerning, because these infections are associated with longer duration of illness, and a greater risk of invasive disease or death. Adverse events increased six-fold within 30 days of infection and three-fold within 90 days, when patients were infected with quinolone-resistant compared to quinolone-susceptible campylobacter strains (Desenclos, JC and Guillemot, D, 2004; Helms, M *et al.*, 2005; Smith, KE *et al.*, 1999). (Helms, M *et al.*, 2005) reported that adverse events increased six-fold within 30 days of infection and three-fold within 90 days, when patients were infected with quinolone-resistant as opposed to quinolone-susceptible *Campylobacter* strains. The evidence for a significant or added risk on public health of FQ resistance in *Campylobacter* is unclear. A meta-analysis of all such studies found no association (Wassenaar, TM *et al*., 2007).

8.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species.

A number of case-control studies have specifically addressed risk factors for FQ-resistant *Campylobacter*. Examples include those provided by (CSSSC, 2002; Engberg, J *et al.*, 2004; Johnson, JY *et al.*, 2008; Kassenborg, HD *et al.*, 2004; Nelson, JM *et al.*, 2004; Smith, KE *et al.*, 1999). All of these case-control studies identified foreign travel as a risk factor for acquisition of a FQ-resistant campylobacter infection. In most of the studies, it was not possible to conclusively say what the exposure food-stuff/route might have occurred when travellers visited these countries, although the *Campylobacter* sentinel study identified consumption of chicken and bottled water as risk factors for travel-related cases. Risk factors for non-travel related cases were as follows: use of a FQ before the collection of the stool specimen (Smith, KE *et al.*, 1999); consumption of cold meat (precooked) (CSSSC, 2002); consumption of fresh poultry other than chicken and turkey (Engberg, J *et al.*, 2004); swimming (pool, ocean, lake or other places) (Engberg, J *et al.*, 2004); consumption of chicken or turkey cooked at a commercial establishment (Kassenborg, HD *et al.*, 2004); and possession of non-prescribed antimicrobials (Johnson, JY *et al.*, 2008).

In Norway, the prevalence of FQ resistance among *C. jejuni* from imported and indigenous sporadic human cases of campylobacteriosis and from domestic broilers was assessed (Norstrom, M *et al.*, 2006). Among the imported human isolates, 67% were resistant to ciprofloxacin compared with 6% of indigenous human isolates. No quinolone preparations are licensed for use in broilers in Norway.

Acke, E, McGill, K, Quinn, T *et al.* (Acke, E, McGill, K, Quinn, T *et al.*, 2009) recently reported a study of 51 *C. jejuni* isolates recovered from cats and dogs presenting at a veterinary hospital in one MS. Over half of the collection was found to be resistant to nalidixic acid and ciprofloxacin. On this basis it was concluded that companion animals should be considered as a potential source of both MDR and ciprofloxacin-resistant *Campylobacter.*

8.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists.

There are no available data to evaluate a connection between the use of quinolone antimicrobials in humans and the emergence or increase in resistance to this class of antimicrobial in *Campylobacter.*

8.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists.

A temporal association between the emergence of quinolone resistance and its increase in isolates both from animals and humans following the introduction of this class of antimicrobial in animal production has been shown by several studies (Engberg, J *et al.*, 2001; Gaudreau, C and Gilbert, H, 2003; Hein, I *et al.*, 2003; Lucey, B *et al.*, 2002).

In a study by Rosengren and colleagues (Rosengren, LB *et al.*, 2009) 10% of 405 porcine faecal *Campylobacter* isolates were resistant to FQs (ciprofloxacin). The strains originated from 20 Canadian grower-to-finishing pig

herds, and analysis revealed quinolone resistance to be negatively correlated with the exposure to (orally) administered β-lactams.

Despite the overall comparability of FQ resistance between animal and human *Campylobacter* strains (Rozynek, E *et al.*, 2008), risk analyses on the consequences for human health are controversial. While some studies have highlighted an increased risk leading to the ban of FQ use in broilers in the USA (FDA 00N-1571, http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064804cbe3d), other warn of adverse public health effects when quinolone use in livestock would be restricted due to a raise in bacterial diseases incidence in broilers and swine production, with an subsequent increase of bacterial load throughout the food chain (Cox, LAJ and Popken, DA, 2006). Short temporal follow-up studies showed a persistence of *Campylobacter a*nd ciprofloxacin-resistant *Campylobacter* in conventional poultry products (Price, LB *et al.*, 2007) and on retail raw chicken carcasses (Nannapaneni, R *et al.*, 2009) a few years after the cessation of FQ use in poultry production in the USA.

8.8. To what extent alternative antimicrobials are available to prevent or treat animal disease.

See 7.8 above.

9. Cephalosporin resistance in *Salmonella.*

9.1. Brief description of the mechanisms of resistance.

The main mechanism of resistance to cephalosporins is through production of β-lactamase enzymes which hydrolyse the β-lactam ring, thereby inactivating the cephalosporin (enzymatic barrier). The genes coding for these enzymes, of which there are a large number of different types, must be acquired by horizontal transmission from other bacteria since they are invariably absent from naturally-occurring *Salmonella* strains.

There are two broad types of β-lactamase enzyme which have been reported most frequently in *Salmonella* and which confer resistance to third-generation cephalosporins. These are:

1) Extended-spectrum β-lactamases (ESBLs) (e.g. TEM and SHV variants and the CTX-M enzymes). These are class A enzymes in Ambler's molecular classification and are inhibited by clavulanate and hydrolyse oxyimino-cephalosporins but not cephamycins (Livermore, DM and Woodford, N, 2006).

2) AmpC β-lactamases which hydrolyse oxyimino-cephalosporins and cephamycins and are also resistant to clavulanate; they are class C enzymes in Ambler's molecular classification (Livermore, DM and Woodford, N, 2006).

In addition to these types of β-lactamase, other types have also been reported in *Salmonella*, for example OXA enzymes, which are assigned to a different molecular class (class D) (Antunes, P *et al.*, 2004) and the KPC enzymes carbapenemases which also confer resistance to cephalosporins (Miriagou, V *et al.*, 2003).

Mechanical barriers including impermeability of the bacterial cell wall can also affect the spectrum of resistance that is shown by the *Enterobacteriaceae* and may occur in conjunction with other resistance mechanisms (Livermore, DM and Woodford, N, 2006; Pages, JM *et al.*, 2008). In addition, as described for other *Enterobacteriaceae* efflux pump activity may contribute to β-lactam resistance (Nagano *et al.*, 2009; Pages, JM *et al.*, 2009).

9.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent.

Meaningful comparison data on cephalosporin resistance in *Salmonella* from animals, food and humans is not feasible at present, due to the many differences that exist in assessing the data itself. Differences in methods and

discrepancies in data collection in AMR testing, in reporting procedures and possibly also in the lack of establishing epidemiological links between the three sources of bacteria, make a comparison not meaningful.

In Quebec, Canada the Québec chicken hatcheries implemented a voluntary withdrawal of the extra-label use of ceftiofur in February 2005. After the withdrawal, a significant decrease in ceftiofur-resistance was seen in *S.* Heidelberg isolates from retail chicken and humans, as well as in *E. coli* from retail chicken (http://www.phac-aspc.gc.ca/cipars-picra/heidelberg/heidelberg-eng.php).

Comparisons made between the prevalence of resistance to cephalosporins in *Salmonella* from animals, food and humans without taking into account the different mechanisms that may confer such resistance may be misleading. Ideally, the same mechanism of resistance at least should be demonstrated in animal, food and human isolates of the same serotype to confirm that the isolates may be epidemiologically linked. The available prevalence data do not always provide this level of detail and whilst broad comparisons may still be made, there is scope for results to be misleading unless further testing is performed. In evaluating resistance testing, problems arise from the use of different breakpoints in the testing and interpretation of the results. This has only recently been resolved in animals by the adoption of guidelines for harmonised monitoring developed by EFSA (EFSA, 2007c).

Testing for cephalosporin resistance in *Salmonella.*

Enter-net and the EFSA Community reports have reported resistance to cephalosporins in human cases of *Salmonella* based on the use of using cefotaxime, a third-generation cephalosporin which is predictive of resistance in ceftriaxone. In contrast, the third-generation cephalosporin ceftiofur has been frequently used by many MS in their veterinary monitoring programmes prior to adoption of the EFSA guidelines. Ceftiofur has recently been found not to be a reliable antimicrobial for the detection of important mechanisms conferring resistance to third-generation cephalosporins. Resistance to ceftiofur correlates with resistance to ceftriaxone and both indicate resistance to third-generation cephalosporins as a group (HPA; NARMS, 2006).

The conclusions that can be drawn from these EU ceftiofur resistance data in meat and food-producing animals are therefore limited and results from human isolates, and from animal and food isolates may not be directly comparable.

Because the prevalence of resistance to third-generation cephalosporins in *Salmonella* from animals is currently low in all MS, it is not possible to provide information on trends in populations with confidence. The human, animal and food prevalences and reports of linkages between epidemiological groups show that transfer along the food chain can occur. The EU picture is also affected by global food imports and also human travel-associated exposure to *Salmonella.* Notwithstanding these considerations, it seems safe to conclude from published reports and from the data submitted to EFSA, that the overall prevalence of resistance to third-generation cephalosporins in *Salmonella* in EU MS is low.

9.2.1. Prevalence data animals/food – humans

Isolates from humans

The emergence of MDR *Salmonella* with additional resistance to third-generation cephalosporins, such as *S.* Typhimurium DT104 and *S.* Newport-MDR-AmpC, is responsible for most of the increase in cephalosporin resistance in the EU and elsewhere (DuPont, HL, 2007; Gupta, A *et al.*, 2003; Threlfall, EJ, 2000)). In Europe and North America, *S.* Enteritidis PT4 has also been reported as MDR with increasing frequency (Gupta, A *et al.*, 2003; Threlfall, EJ, 2000; Whichard, JM *et al.*, 2007).

In the EU, much variation exists amongst the MS in the reporting of *Salmonella* from cases of human infection making it difficult to follow trends in resistance. Data from the EFSA Community Report, show an overall EU cefotaxime resistance of 0.1% for S. Enteritidis and 0.6% for S. Typhimurium in 2005, remaining stable in 2006 with 0.1% in *S.* Enteritidis and 0.9% in *S.* Typhimurium. In 2006, multidrug resistance (defined as resistance ≥ 4 antimicrobials) was 40% in *S.* Typhimurium, but only 0.7% in *S.* Enteritidis (EFSA, 2007a).

Isolates from animals and food

Different national surveys performed in Germany (Rodriguez, I *et al.*, 2009), Italy (Chiaretto, G *et al.*, 2008), the UK [http://www.defra.gov.uk/], Denmark (Aarestrup, FM *et al.*, 2006), Norway (http://www.vetinst.no) and Spain (Riano, I *et al.*, 2009), and collected data from EU MS (EFSA, 2006, 2007a, 2007b and from a EFSA-funded study undertaken by the Danish Technical University (DTU) (EFSA, 2009) have demonstrated that resistance to broad-spectrum cephalosporins is low among *Salmonella* from animals/food products in northern MS although higher in southern and eastern European countries. Nevertheless, a continuous increase in this prevalence is observed in several countries, such as the Netherlands, Denmark and France, mainly linked to the spread of clonal lines, namely *S.* Typhimurium, *S.* Java and *S.* Agona.

The resistance to broad spectrum cephalosporins is mainly associated with ESBL-producing *Salmonella* of different serovars, although AmpC-type is raising in different European countries, CMY-2 being the most widely disseminated of these enzymes (Rodriguez, I *et al.*, 2009).

Salmonella resistant to extended-spectrum cephalosporins is predominantly associated with poultry and poultry meat products (Arlet, G *et al.*, 2006; Chiaretto, G *et al.*, 2008; MARAN, 2007), although also described in humans, other animals and food products.

EFSA have recently reviewed the susceptibility data submitted to them by EU MS as part of national monitoring for *Salmonella* from food animals and food conducted over the period 2004-2007 (EFSA, 2009). For *Salmonella* from chickens there were 1388 isolates tested for ceftiofur resistance from Austria, Denmark, Germany, Hungary, Poland and Slovenia and of these 1% of isolates from Hungary (n=153) were resistant in 2006. There were 119 isolates from turkeys in Germany and resistance to ceftiofur ranged from 2-5%; 20 isolates from Slovenia were susceptible. Isolates from pigs in Denmark (n=3210), Germany (n=957), Hungary (n=19), Slovenia (n=22) and Sweden (n=46) were all susceptible to ceftiofur. Isolates from cattle in Denmark (n=97) and Sweden (n=67) were all susceptible to ceftiofur. Isolates from cattle in Germany in 2004, 2005 and 2007 were susceptible to ceftiofur (n=480) but 1% of 194 isolates from cattle in Germany in 2006 were resistant. Considering ceftiofur resistance in *S.* Typhimurium and *S.* Enteritidis, for *S.* Enteritidis from *Gallus gallus* Hungary reported resistance to ceftiofur in 6% of isolates in 2006 (total number of Enteritidis isolates examined by Hungary =18). In *S* Typhimurium 1% of 163 isolates from cattle in Germany were resistant to ceftiofur in 2006.

Considering the monitoring performed on meat and the quantitative data reported, isolates from Hungary (n=20) and Switzerland (n=25) were tested in 2006 and 2007 respectively from broiler meat and no resistance to ceftiofur was detected. Germany reported that 1% of 214 isolates from broiler meat were resistant to ceftiofur in 2007. No resistance t was detected in isolates from beef in 2006, 2007 (n=21). Denmark did not report ceftiofur resistance in 178 *Salmonella* isolates from pork over the period 2004-2007; Germany did not report ceftiofur resistance in isolates from pork in 2005 (n=281) or 2006 (n=118) though 1% of 117 isolates were resistant to this antimicrobial in 2007.

Quantitative antimicrobial susceptibility data has also been reported by some MS in this monitoring. In Belgium 5 % of *Salmonella* isolates (n=621) in 2005 and 9 % of isolates in 2006 (n=583) from chickens were resistant to ceftiofur; Poland examined 354 isolates from turkeys in 2004 and 7% were resistant. Belgium examined 395 isolates from pigs in 2004 and 1% were resistant, whilst none of 271 tested in 2006 were resistant. No resistance was detected in 148 isolates from cattle in Belgium in 2004 / 2006.

The conclusions that can be drawn from the above EU ceftiofur resistance data in meat and food-producing animals are limited because ceftiofur is not an ideal indicator cephalosporin for detecting important mechanisms of third-generation cephalosporin resistance (see above). There are also issues relating to the variability of the test methods used (the available data pre-dates EFSA's harmonised guidelines), including the breakpoints selected (EFSA, 2007c). Additionally, not all countries have reported data. Notwithstanding these considerations, it seems safe to conclude that in general the overall prevalence of resistance to third-and fourth-generation cephalosporins in *Salmonella* in EU MS is in general low or very low. The serotypes with resistance to third-generation cephalosporins should be named in national monitoring programmes to facilitate identification of emerging trends and comparisons of data from animals, food and humans.

9.2.2. Comparison between those prevalences; their significance

When comparing AMR data from animals, food and humans, there area number of issues involved that can impact on the comparability. A large proportion of the food consumed might be imported, which can result in large differences between the reported occurrence of AMR in isolates from food and animals. Several different types of food are often possible sources of human salmonella infections. In addition, the occurrence of AMR among *Salmonella* from different animal species and food sources may vary considerably within the same country, which can make it difficult to find associations between the occurrence of AMR in food and human salmonella isolates.

As discussed above, ceftiofur has recently been found not to be a reliable antimicrobial for detection of the various types of mechanisms conferring resistance to third-generation cephalosporins, although in recent years it has been used for testing by many EU countries in their veterinary monitoring programmes. The usefulness of the ceftiofur resistance data is therefore limited. For that reason, selected examples have been chosen which have been reported by EU member states which illustrate particular facets of the issue. Countries vary in the amount of resources they can deploy to provide surveillance on AMR and countries with rudimentary surveillance programmes may have undetected links between the resistance isolates occurring in animals, food and man.

The data available from the Netherlands (MARAN, 2007) strongly suggest that transmission of *S.* Java resistant to third-generation cephalosporins can occur via the food chain, with the resistant microorganism detected in broilers, raw broiler meat and in a single human. The significance of the findings is increased by the occurrence of concomitant ciprofloxacin resistance in *S.* Java, because the isolates have resistance to two of the antimicrobials which could be considered in first-line antimicrobial treatment. The background of third-generation cephalosporin resistance in commensal *E. coli* in broilers in the Netherlands has been suggested as the reservoir of resistance which is acquired by *S.* Java isolates. This reinforces the value of monitoring resistance in commensal *E. coli*. Similar findings have been reported relating to a cephalosporin-resistant *S.* Virchow clone in Belgium which was found throughout the food chain. A similar spread was demonstrated for cephalosporin-resistant *S.* Infantis. In this case, ESBL resistance was located on a conjugative plasmid that had already spread to some other serotypes, including Java and Typhimurium (Bertrand, S *et al.*, 2006; Cloeckaert, A *et al.*, 2007).

Because the prevalence of resistance to third-generation cephalosporins in *Salmonella* from animals is currently low in all EU countries, it is not possible to provide information on trends in populations with confidence. The human, animal and food prevalences and reports of linkages between epidemiological groups show that transfer along the food chain can occur. One would expect that trends in resistance in isolates from the animal population would lead to concomitant trends in antimicrobial-resistant bacteria in food and consequently in humans, assuming that the current production, processing and food distribution systems and factors relating to those remain constant.

The EU picture is affected by global food imports and also human travel-associated exposure to *Salmonella.* It is therefore important to identify and separate those different sources where possible.

In *Salmonella* resistance to broad-spectrum cephalosporins has resulted from the acquisition of different β-lactamase genes (e.g., CTX-M-9, CTX-M-2, CTX-M-32, TEM-52, SHV-12, CMY-2). Such resistance is almost invariably plasmid-mediated and has been frequently been associated with the clonal spread of both plasmids and resistant serovars. As the majority of plasmid-encoded ESBL and AmpC genes are located on conjugative plasmids (Canton, R *et al.*, 2008; Carattoli, A, 2009), molecular characterization of clones, plasmids and genes associated to cephalosporinases should be conducted in addition to monitoring the prevalence of cephalosporin resistance.

9.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

Resistance to third- and fourth-generation cephalosporins in *Salmonella* is primarily caused by production of ESBLs, e.g. with substrate specificity depending on the mechanism and sequential mutations involved. Examples are TEM, SHV, and OXA families, although some only confer resistance to first-generation cephalosporins. Originally restricted to the chromosome, genes encoding AmpC-type β-lactamases are increasingly associated with plasmids. In particular, in different *Salmonella* serovars the enzyme CMY (cefamycinase) has often been

identified on plasmids (EMEA, 2009). Plasmid-mediated ESBLs and AmpC type resistances are frequently found together with determinants conferring resistance to other antimicrobials, e.g., aminoglycosides, chloramphenicol and florfenicol, sulphonamides, tetracycline and/or trimethoprim (EMEA, 2009) leading to efficient spread via co-selection. Both ESBLs and AmpC confer resistance to extended-spectrum cephalosporins and other β-lactam antimicrobials. An exhaustive list of the β-lactamase families with their substrate specificity is provided elsewhere (EMEA, 2009).

There are no data available to evaluate a connection between the use of biocides and the emergence or increase in resistance to cephalosporins in *Salmonella.*

9.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections

The most recent available data on cephalosporin resistance in *Salmonella* from the EU are from 2006. The incidence of resistance of *S.* Typhimurium and *S.* Enteritidis to cefotaxime was 0.9% and 0.1%, respectively. Multidrug resistance was reported in 40% of *S.* Typhimurium and 0.7% of *S.* Enteritidis isolates (EFSA, 2007b).

There are only limited data on outcomes of human infections with cephalosporin-resistant *Salmonella,* although; MDR salmonella infections have been shown to result in worse outcomes than infections with susceptible *Salmonella* (Helms, M *et al.*, 2002; Martin, LJ *et al.*, 2004).

9.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species

In 1984, a strain of *S.* Newport with resistance to cephalosporins originating in cattle in the USA was traced through the food chain to humans (Holmberg, SD *et al.*, 1984). In a US FoodNet case-control study of sporadic MDR *S.* Newport infections, (Varma, JK *et al.*, 2006) concluded that patients were more likely to have consumed uncooked ground beef or runny scrambled eggs or omelettes prepared in the home. Travel was not a risk factor for infection with multiple-resistant *S.* Newport.

The transmission of broad-spectrum cephalosporin resistance in *Salmonella* to humans, either through the food chain or by direct contact between humans and animals has been conclusively demonstrated on only a few occasions. In the USA a ceftriaxone-resistant strain of *S.* Typhimurium which caused an infection in a child was linked to an outbreak in cattle on his father's farm. In EU MS the prevalence of resistance to third-generation cephalosporins in food-producing animals and meat appears to be low or very low, based on the available data from EFSA. The data are not comprehensive and there are problems in making direct comparisons, as the data pre-dates harmonised monitoring guidelines introduced in 2007 and are not harmonised or optimised for the detection of third-generation cephalosporin resistance.

ESBL resistance has recently been detected in many countries worldwide in various serotypes of salmonella strains exhibiting such resistance have been detected in both humans and animals in Europe (Bertrand, S *et al.*, 2006; EMEA, 2009; Riano, I *et al.*, 2009). In Belgium and France, a cephalosporin-resistant *S.* Virchow clone (carrying CTX-M-2) was found in poultry, poultry products and humans in 2000-2003. Two human patients who contracted this clone were initially treated unsuccessfully with extended-spectrum cephalosporins, confirming the clinical significance of third-generation cephalosporin resistance. All isolates belonging to this clone of *S.* Virchow also displayed decreased susceptibility to ciprofloxacin. The chronology of isolation suggested that the strain had been transmitted to humans by the food chain, probably by poultry meat. A similar spread was demonstrated for a clone of cephalosporin-resistant *S.* Infantis in poultry and humans in Belgium and France over the period 2001-2005. In this case, ESBL resistance (TEM-52) was located on a conjugative plasmid which also spread to some other serotypes, including Java and Typhimurium (Bertrand, S *et al.*, 2006; Cloeckaert, A *et al.*, 2007). The authors commented that human infections with cephalosporin-resistant *S.* Infantis were probably related to ingestion of undercooked poultry products. There have been numerous reports of resistance to resistance to cephalosporins mediated by CTX-M enzymes in salmonella infections in humans in many countries outside the EU, but in general such infections have not been linked to food production animals.

In relation to human infection and domestic pets, a study in Canada, found an association between handling pet treats containing dried beef and human infection with *S.* Newport expressing the *amp*C β-lactamase CMY-2, which confers resistance to third-generation cephalosporins (Pitout, JD *et al.*, 2003). No salmonellas were recovered from stools taken from the pets receiving these treats in affected households; salmonella isolates from affected human patients were highly related to an isolate recovered from one of the commercial pet treats.

9.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists.

There are no data to support a connection between the use of antimicrobials in humans and the emergence or increase of AMR, including cephalosporin resistance, in *Salmonella* in the EU.

9.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists.

Studies in cattle and swine have established a link between cephalosporin administration, including treatment frequency, and resistance selection in *E. coli* (EMEA, 2009). *In vivo* transfer to, as well as presence of, many of these ESBL genes in *Salmonella* has been demonstrated in several studies (EMEA, 2009).

9.8. To what extent alternative antimicrobials are available to prevent or treat animal disease.

For almost all of the indications for which ceftiofur or cefquinome are authorised for systemic therapy in food producing animals, alternatives are available. For example for streptococcal infections, cephalosporins have generally no advantage above benzylpenicillin in terms of antimicrobial efficacy or safety. In cattle, the only indication in which third- or fourth-generation cephalosporins could be the sole treatment option is severe clinical mastitis with life-threatening sepsis caused by *Enterobacteriaceae* such as *E. coli* or *Klebsiella*. Cephalosporins are poorly distributed to the milk compartment, and their systemic use would be rational only in septic mastitis. The few antimicrobials that have shown some beneficial effect in therapy of severe coliform mastitis are fluoroquinolones and third- or fourth-generation cephalosporins (Erskine, RJ *et al.*, 2002; Poutrel, B *et al.*, 2008; Rantala, M *et al.*, 2002; Shpigel, NY *et al.*, 1997). In horses, the only indication where cephalosporins can be regarded as critically important is neonatal sepsis in foals. In the treatment of this condition, penicillin-aminoglycoside or penicillin-trimethoprim-sulphonamide combinations are listed as 'first choice' in standard textbooks (Giguère, S, 2006; Weese, JS *et al.*, 2008). In many countries, resistance to both gentamicin and trimethoprim-sulphonamides in the Gram-negative target pathogens exist. In such cases, third- or fourth-generation cephalosporins could be the only effective alternatives.

In conclusion, in most cases the direct impact of infections resistant to cephalosporins on animal health is low. The emergence of resistance mediated by genes encoding ESBLs or AmpC among *Salmonella* and *E. coli* is frequently linked to resistance to other antimicrobials. A further increase of cephalosporin resistance can indirectly impact on animal health by increasing the prevalence of multidrug resistance, thereby severely reducing the number of effective alternatives for treatment (EMEA, 2009).

10. Macrolide resistance in *Campylobacter*.

10.1. Brief description of the mechanisms of resistance.

Macrolide compounds inhibit bacterial growth by binding to the 70S ribosome blocking protein synthesis. Generally the mechanisms of resistance (Payot, S *et al.*, 2006) can be divided into three groups: (a) modification of the antibiotic through the activity of esterases and/or phosphotransferases-a mechanism that has not been described in *Campylobacter*; (b) modification of the antibiotic target site via mutation or methylation and (c) extrusion of the antibiotic from the bacterial cell by efflux pumps, e.g. the RND pump CmABC (Luangtongkum, T *et al.*, 2009).

The 14-C member antibiotic erythromycin binds to the 23S rRNA in the vicinity of the peptidyl-transferase centre (PTC). Erythromycin or other macrolide analogues do not inhibit peptide bond formation per se, but block entrance of the nascent chain to the peptide exit tunnel. This allows for the synthesis of short nascent peptides also in the presence of macrolides, where the maximal peptide length is defined by the space available for peptide growth between the macrolide and the PTC (Tenson, T *et al.*, 2003).

Modification of the macrolide ribosomal targets is the most common resistance mechanism encountered in *Campylobacter* spp. This occurs via mutation. Two nucleotides close to each other are target sites for modification. Mutation of A2075G results in a high-level erythromycin resistance (MIC > 128 mg/ml) in clinical strains of *C. jejuni* and *C. coli*. Since multiple copies of these genes exist, often a mosaic of resistance can be described, wherein not all targets are modified. A2074C or A2074T transversion mutations were described in a clinical isolate of *C. jejuni* associated with an MIC > 128 mg/ml (Gibreel, A *et al.*, 2005).

Mutations affecting the ribosomal proteins L4 and L22 were also identified (Corcoran, D *et al.*, 2006). These were associated with both *C. jejuni* and *C. coli* that possessed a A2075G polymorphism in the 23S rRNA gene. A number of different mutations have been described in both ribosomal protein-encoding genes (Gibreel, A *et al.*, 2005). In some isolates, the target mutations are associated with the expression of active efflux pumps that contribute to the resistance level (Mamelli, L *et al.*, 2005, 2007).

10.2. To what extent the prevalence of resistance to different antimicrobials is comparable between animal/food thereof and human isolates of the agent.

10.2.1. Prevalence data animals/food – humans

Isolations from humans

Campylobacter infection usually results in enteroinvasive diarrhoea. Most cases of campylobacteriosis are self-limited and do not require antimicrobial therapy. When therapy is required, macrolides have commonly been used as the first-line drug for campylobacter enteritis (Guerrant, RL *et al.*, 2001), although FQs have also been widely used for this indication.

In the EU, campylobacter infection has been the most commonly reported zoonotic illness from 2004-2007. In 2004, resistance to erythromycin in human campylobacter isolates was reported to Enter-net by only seven MS and was only available for *Campylobacter* spp. and not by species. The lowest reported percentage of resistance was 0.9% in Lithuania and the highest, 7% in Belgium. The EFSA Community Report states that, in the EU in 2006, 2.3% of all *C. jejuni* and 10% of *C. coli* were resistant to erythromycin. Multidrug resistance, defined as resistance to ≥4 antimicrobials, was reported in 8% of *C. jejuni* and 17% of *C. coli* (EFSA, 2007b). No comparable data were reported from 2004 or 2005.

Isolates from animals

Campylobacter organisms are widespread in nature. The recognized reservoirs of this bacterium are the alimentary tracts of wild and domesticated birds and mammals. In particular *Campylobacter* are prevalent in poultry, cattle, pigs, sheep and in companion animals including dogs and cats. Other sources include wild birds and environmental water. Animals rarely show signs of disease associated with these pathogens.

Compared to the data for quinolones, resistance to erythromycin in *Campylobacter* from 2004 through 2007 (EFSA, 2009) was generally low ranging from 0% to 13% for *C. jejuni*. Three MS reported higher levels of resistance in *C. jejuni* from *Gallus gallus*.

When compared with *C. coli*, levels of resistance to this antimicrobial were higher, with the highest occurrence being reported from pigs, with variations noted between countries. In examining the trends, the occurrence of resistance appeared to be s increasing in some countries whilst decreasing in others.

In general *C. coli* would appear to be more resistant to macrolides than compared to *C. jejuni*. This observation is in accordance with the published literature (Payot, S *et al.*, 2006). As most isolates have been recovered from

pigs, this finding may reflect the chemotherapeutic choice made by veterinary practitioners in managing infections in these food-producing animals.

Various case-control studies including a recent one by Danis and colleagues (Danis, K *et al.*, 2009) highlight the risk associated with the consumption of chicken. Campylobacter-associated enteritis is an important cause of morbidity across the globe (Rozynek, E *et al.*, 2008) and human exposure *via* retail chicken (Gormley, FJ *et al.*, 2008) including the types of *Campylobacter* involved require careful delineation. Poultry can act as a reservoir to transmit antimicrobial-resistant *Campylobacter* to humans (Rozynek, E *et al.*, 2008).

A pan-European study involving five MS using CLSI breakpoints and decreased susceptibility limits based on EFSA epidemiological cut-off values, reported that in *C. jejuni* clinical resistance was absent in isolates cultured from chickens and cattle (de Jong, A *et al.*, 2009) and decreased susceptibility was low. Similar trends were observed for *C. coli.* Comparing macrolide resistance between *C. jejuni* and *C. coli* isolates in south-eastern Italy using disk diffusion, Parisi *et al* reported 3% and 23% erythromycin resistance in *C. jejuni* and *C. coli* respectively from poultry and 4% erythromycin resistance in *C. jejuni* cultured from cattle (Parisi, A *et al.*, 2007). In the latter study these authors conclude that there is a different propensity between *C. jejuni* and *C. coli* to become resistant to this antimicrobial.

In Turkey, (Bostan, K *et al.*, 2009) the susceptibilities of 246 isolates were determined using a small panel of antimicrobials. In this report 57% of the isolates were resistant to erythromycin. A Czech study comparing isolates from poultry and humans by microdilution, reported higher resistance among *Campylobacter* from animals, with the latter showing 6% resistance to erythromycin compared to 1% for human isolates (Bardon, J *et al.*, 2009). In bivalve molluscs harvested in Thailand, 72-84% were resistant to erythromycin by E-test, highlighting the importance of aquaculture as a reservoir for these antimicrobial-resistant pathogens (Soonthornchaikul, N and Garelick, H, 2009).

Based on recent data from ECDC reporting human outbreak cases of *Campylobacter* in 2006, the prevalence of erythromycin resistance ranged from 0% to 14% among eight MS.

10.2.2. Comparison between those prevalences; their significance

Comparison between resistance in *Campylobacter* in the EU between animals, food and humans is difficult, as methods of testing and reporting by MS are not standardised.

Campylobacter is one of the most common aetiological agents of food-borne illness (Horrocks, SM *et al.*, 2009). The prevalence of this zoonotic pathogen can exceed 80%, imposing significant pressure on pre- and post-harvest reduction measures. It has been suggested that cleaning and disinfection measures applied in slaughter-houses might select for strains, especially where those resistance mechanisms are shared. Strains that are adapted to this environment may have a selective advantage, facilitating their continued survival and thus requiring a better understanding of the pathogen infection dynamics (Skanseng, B *et al.*, 2007) as an important step towards their elimination.

In the UK poultry meat was more frequently contaminated with *Campylobacter* (at a level of 53%) compared to *Salmonella* (7 %), with chicken meat exhibiting the highest levels of contamination (Little, CL *et al.*, 2008b). In the latter study *C. coli* were more likely to exhibit AMR compared to *C. jejuni.*

10.3. Has cross-resistance with other antimicrobials been demonstrated? If so, with which antimicrobials?

For macrolides, resistance selection by other antimicrobials is very common if *erm* (X) genes - conferring resistance to macrolides, lincosamides and B-compounds of the streptogramins – are present the so called MLS_B phenotype. Generally this is mediated by *erm*-encoding methylases that modify the A2058 nucleotide (*E. coli* numbering) in the 23S rRNA. As earlier mentioned, this mechanism has however not yet been described in the *Campylobacter* species of major zoonotic importance (Payot, S *et al.*, 2006).

The mutations in the 23S rRNA target gene (domain V) often confer high-level macrolide resistance in *C. jejuni* and *C. coli* for the older macrolide groups such as erythromycin, azithromycin, tylosin whereas ketolides

(telithromycin, tulathromycin) may be less affected (Cagliero, C *et al.*, 2005). Thus macrolide-resistant *Campylobacter* are resistant to macrolides used in human medicine, such as erythromycin, azithromycin and clarithromycin.

In *Campylobacter*, the RND pump CmeABC is known to contribute to intrinsic and acquired resistance to macrolides fluoroquinolones, and ß-lactams in *C. jejuni* and *C. coli* (Corcoran, D *et al.*, 2005; Gibreel, A and Taylor, DE, 2006; Gibreel, A *et al.*, 2007; Luangtongkum, T *et al.*, 2009; Quinn, T *et al.*, 2007). In addition to its role in mediating resistance to antimicrobials, the CmeABC pump also mediated resistance to bile salts, a key virulence feature. Although not the antimicrobial of choice for severe campylobacter infections, resistance to cephalosporins is mostly not present even when β-lactamase (bla_{OXA61}) is found (Griggs, DJ *et al.*, 2009).

There are no data available to support a connection between the use of biocides and the emergence in increase of resistance to macrolides in *Campylobacter*.

10.4. The burden of disease of resistant infections in humans, e.g. in comparison with sensitive infections

Direct data comparing infections due to macrolide-resistant and macrolide-susceptible isolates are not available. Resistance to macrolides causes delay in appropriate treatment, treatment failures and need for alternative antimicrobials. Infections with macrolide-resistant *Campylobacter* are associated with an increased frequency of adverse events, invasive illness and death compared to susceptible infections (Helms, M *et al.*, 2005; Travers, K and Barza, M, 2002).

Helms *et al.* (Helms, M *et al.*, 2005) reported that erythromycin-resistant *Campylobacter* isolated were associated with an increased risk of adverse events of 9.68-fold within 30 days and 5.5-fold within 90 days after infection, as compared to susceptible isolates. Similar results were also reported by Engberg *et al.* (Engberg, J *et al.*, 2004), showing the human health consequences of resistance to clinically-important antimicrobials among *Campylobacter* infections and the need for increased efforts to mitigate such resistance.

In 2006 2.3% of all *C. jejuni* and 10% of *C. coli* were resistant to erythromycin and multidrug resistance, defined as resistance to ≥4 antimicrobials, was reported in 8% of *C. jejuni* and 17% of *C. coli* isolates. Infections with macrolide-resistant *Campylobacter* have been associated with an increased frequency of adverse events, including invasive illness and death compared to susceptible infections.

10.5. To which extent humans are exposed to the resistance agent through food or contact (e.g. pets) with the relevant species.

In a study from Korea, 770 retail raw meat samples were investigated for MDR *Campylobacter*. Data from this study showed the widespread nature of the microorganism and that resistance to erythromycin (14%) was relatively common. (Hong, J *et al.*, 2007).

(Levesque, S *et al.*, 2007) compared *C. jejuni* isolates from humans, with those from various foods, including chicken, raw milk and the environment. In the latter study 16% of isolates from chickens were resistant to erythromycin.

A recent study of *C. jejuni* isolates from cats and dogs found that 12% of isolates were resistant to erythromycin. In one MS campylobacter isolation rates from cats and dogs of 75% and 88% respectively have been reported (Acke, E *et al.*, 2006). Onward transmission from these sources to humans is a recognised risk for contracting campylobacteriosis in humans (Damborg, P *et al.*, 2004; Tenkate, TD and Stafford, RJ, 2001). Exposure to contaminated food may be an important factor in the dissemination of this antimicrobial-resistant pathogen (Acke, E, McGill, K, Golden, O *et al.*, 2009).

10.6. To which extent a link between the use of antimicrobials in humans and the emerging/increase of AMR in humans exists.

There are no available data to evaluate a connection between the use of macrolide antimicrobials in humans and the emergence or increase in resistance to this class of antimicrobial in *Campylobacter.*

10.7. To which extent a link between the use of antimicrobials in animals and the emerging/increase of AMR in humans exists.

In a Canadian study examining the resistance patterns of porcine *Campylobacter*, over 70% were resistant to macrolides (Rosengren, LB *et al*., 2009). Risk analysis revealed a clear association between the (oral) administration of macrolides and the presence of resistance in faecal isolates. There is controversy regarding the public health aspects of macrolide- resistance in *Campylobacter*, with estimates based on a recent risk analysis not exceeding 1 out of 49,000 impaired human treatments in cases of infection with macrolide-resistant *C. coli* of porcine origin (Hurd, HS and Malladi, S., 2008). The risk for suboptimal treatment due to macrolide-resistant *C. jejuni* infections from broiler and bovine sources even was lower.

10.8. To what extent alternative antimicrobials are available to prevent or treat animal disease.

A recent study investigating the effect of cleaning and disinfection procedures in poultry slaughterhouses on the development of or selection for biocide and AMR in *C. jejuni* and *C. coli* showed that a very low number (1-2) of genotypes were recovered after cleaning and disinfection. There was no increase in AMR before and after exposure to the disinfection procedures (Peyrat, MB *et al.*, 2008).

Macrolides are primarily used to control gastrointestinal disorders in pigs and have a limited use for treatment of bovine mastitis. Furthermore, a number of new macrolides are used for treatment of respiratory infections. In most cases alternatives exist.

11. Areas where innovation and research should be encouraged.

11.1. Improvement of surveillance activities and risk assessment

The needs for consideration of the best ways of ensuring harmonisation/standardisation of AMR data collected in all MS, including data from cases of human infection, include:

- Sensitivity and specificity analyses of isolation, identification, and susceptibility testing methodology should be improved to increase the risk/benefit and cost/benefit of monitoring.
- Development of unified methods of collection of antimicrobial usage data (including biocides) in both the veterinary and human areas in all MS. And other relevant biosecurity conditions.
- Development of surveillance programmes to monitor the level of resistance and cross-resistance of environmental isolates in all areas of biocide usage, in particular the health care setting, veterinary setting and food industry.
- Improving detection and sub-typing methodology of relevant micro-organisms and molecular sequences of resistance genes, including identification of mechanisms of resistance, in order to increase the speed of diagnosis.
- Research to strengthen the power of sampling strategies.
- Exploration of the origin and transmission of ESBL-producing *E. coli* through the food chain.

- Development of strategies to explore the occurrence of resistance in non-pathogenic commensal micro-organisms (e.g. *E. coli*), together with their ability to develop, harbour, and transmit resistance genes.

- Structured surveillance of animal target pathogens, and the resistance genes/mechanisms therein.

- Risk assessments of the combinations identified. New approaches might be required to perform quantitative risk assessments. In particular, a quantitative risk assessment is needed. to determine the extent to which there is a link between the use of antimicrobials in animals and emerging/increase of quinolone resistance in *Salmonella* from human,

- Identification and characterisation of those environments that facilitate bacterial gene transfer. Initially, this should focus on zoonotic bacteria but should address subsequently other bacteria, such as *E. coli*. In particular the following issues should be considered:

  - Specific environments favouring bacterial gene exchange from pathogenic micro-organisms to environmental and opportunistic ones and *vice versa.*
  - Link between bacterial species and genetic elements or resistance mechanisms favouring the acquisition of additional resistance leading to the emergence of multidrug resistance phenotypes (identification, risk assessment).

11.2. Development and use of antimicrobials

- The development of new antimicrobials and new inhibitors of resistance mechanisms should be encouraged

- The antimicrobial regimen includes the dose, treatment duration, treatment interval, and route of administration (formulation). These variables should be accompanied by appropriate pharmacokinetic/pharmacodynamic studies to minimise the emergence of resistance.

- Longitudinal studies should examine the robustness of currently applied regimens in the long term with respect for the individual patient but also at the population level.

- The development of new classes of antimicrobials should be encouraged, in particular where co-resistance is minimal.

- The comparative efficacy of different antimicrobials and antimicrobial regimes for treatment of different infections in food producing animals.

- Laboratory research and *in-situ* follow-up are necessary for clarifying the eventual link between the use of antimicrobial products including biocides and the selection and dissemination of AR bacteria, and expression of virulence markers. The consequences of pressure of selection induced by chemical use (e.g. antimicrobials, biocides, detergents) should be analysed.

- Antimicrobial monitoring should be used to reveal trends of use of antimicrobials and evaluate the results of management policies set up to reduce such usage.

11.3. Development of new strategies to combat the diffusion of antimicrobial-resistant bacteria and AMR

- Research is required into alternative methods of control of infectious disease in animals other than the use of antimicrobials, such as vaccination, or other methods to interfere or block infectious agent transmission and the development of husbandry methods to reduce AMR.

- Genotyping of micro-organisms with Multidrug resistance phenotypes should be encouraged, including identification of potential virulence determinants. Linkage between the latter and resistance

determinants should be identified and documented. The understanding of the molecular and genetic basis of AMR mechanisms should be encouraged, in order to follow the dissemination and the acquisition of AMR genes by bacterial species involved in zoonotic infections.

- Links between bacterial species and genetic elements or AMR mechanisms favouring the acquisition of additional resistance leading to the emergence of multidrug resistance phenotypes should be identified.

- The contribution of animals/food of animal origin as source/reservoir of epidemic clones/mobile genetic elements carrying resistance and/or virulence traits should be critically evaluated. Such studies should include identification of the main way(s) humans can acquire antimicrobial resistant bacteria from foods (i.e. cross-contamination, insufficient cooking, or survival during cooking and preservation).

- Resistance mechanisms should be identified and characterised by the latest techniques and should cover a broader spectrum than merely antimicrobials.

- Environments that facilitate bacterial gene transfer should be identified ad characterised. Initially such studies should focus on zoonotic bacteria but should subsequently address other bacteria, such as *E. coli*.

- The use of mathematical modelling to guide studies and identify the most optimal point for intervention should be encouraged

11.4. Assess possible contribution of other agents in the selection of antimicrobial-resistant micro-organisms

- Laboratory research and *in-situ* follow-up are necessary for clarifying putative links between the use of antimicrobials and related products including biocides and the selection and dissemination of antimicrobial-resistant bacteria. The consequences of selection pressure induced not only by antimicrobials but also by biocides and detergents should be analysed.

- Standardized methodologies for the evaluation of the capability of a biocide to induce/select for AMR need to be developed.

- The role of antimicrobials in the selection of bacteria that are intrinsically-resistant or that have acquired resistance needs to be clarified.

- Data on the use of biocides in MS should be compiled in conjunction with antimicrobial usage data.

- Surveillance programmes should be developed to monitor the level of resistance and cross-resistance of environmental isolates in all areas of biocide usage, in particular the health care and veterinary settings, and the food industry.

- Bacteria as a source of AMR mechanisms and as a vector of dissemination of AMR genes have to be placed in the core of research efforts, whether they are linked to zoonotic and human infections or not. Genetic mobile elements should be clearly classified and mechanisms of horizontal transmission defined.

- It is important to determine the molecular and genetic aspects which are involved in the emergence and dissemination of bacterial strains exhibiting resistance mechanisms. Clear and well-referenced criteria or standards for the evaluation of the capability of a biocide to induce/select for AMR mechanisms need to be developed.

- The role of bacterial biofilms in the colonisation process, in surviving antimicrobial treatments, and in the sources and dissemination of AMR genes, should be established.

- Considering the high uncertainty in the *in vivo* evaluation of the effects of biocides on the emergence of AMR, reporting of production and use of biocides should be promoted.

RECOMMENDATIONS

This is a series of recommendations that are relevant to zoonotic infections and have been collated from previous reports from the ECDC, EFSA, EMEA[20] and SCENIHR.

Recommendations on quinolones for food-producing animals[21]

- Fluoroquinolones should be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to other classes of antimicrobials. The need of prophylactic use should always be carefully considered and preserved for specific circumstances.
- The dosage regimens of fluoroquinolones should be carefully determined on the basis of their pharmacokinetic and pharmacodynamic properties to ensure optimal efficacy and reduce selection of resistance.
- Veterinarians and farmers should be continuously educated on strategies to minimise antimicrobial resistance
- Emergence of (fluoro)quinolone resistance in pathogenic and indicator bacteria should be monitored and the need for interventions should be continuously evaluated.
- Use of (fluoro)quinolones should be monitored in each country and this should be done by animal species to measure the effect of interventions described above.
- All Member States should implement and enforce internationally recognised code of practice of rational and prudent use of antimicrobials (Codex code of practice to minimize and contain antimicrobial resistance CAC/RCP 61-2005; the OIE terrestrial code – chapter on antimicrobial resistance).

Recommendations on third and fourth generation of cephalosporins for food-producing animals[22]

Although it may be assumed that a large part of the increased incidence of resistance in human medicine is due to comprehensive human usage, and notwithstanding that no full quantitative or qualitative risk assessment of the risk posed by cephalosporin resistant bacteria or resistance determinants has been done the following actions on the veterinary side to reduce the possible risk for veterinary use contributing to emergence of resistance in human pathogens are recommended. Furthermore, action is needed in order to maintain the efficacy of cephalosporin-containing veterinary medicinal products.

- Prudent use of antimicrobials should be strongly promoted.
- For systemically administered broad spectrum cephalosporins (3rd and 4th generation) it should be reflected in the Summary of Product Characteristics (SPC) that these are to be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow spectrum antimicrobials. Increased use, including use of the product deviating from the instructions given in the SPC, may increase the prevalence of bacteria resistant to the relevant antimicrobial. Official, national and regional antimicrobial policies should be taken into account when the product is used.
- Authorisation of products for prophylactic use of systemically administered cephalosporins should always be limited to specific circumstances and carefully considered in the conditions for authorisation and reflected in the SPCs.
- Use of systemically administered cephalosporins for groups or flocks of animals such as use of oral cephalosporins in feed or drinking water should be strongly discouraged, except in very specific situations, and special attention should be given to the risk of antimicrobial resistance as part of the benefit/risk assessment.
- Prudent use guidelines in all countries should take into account risks related to emergence of resistance to cephalosporins and all Member States should take measures to ensure the implementation of such guidelines.
- Off label use should be strongly discouraged.

[20] The EMEA is currently preparing a document that will also provide recommendations on macrolides for food producing animals.
21 Public statement on the use of (fluoro)quinolones in food-producing animals in the European Union: development of resistance and impact on human and animal health (EMEA/CVMP/SAGAM/184651/2005)
22 Revised reflection paper on the use of 3rd and 4th generation cephalosporins in food producing animals in the European Union: development of resistance and impact on human and animal health (EMEA/CVMP/SAGAM/81730/2006-Rev.1)

- Cephalosporins should not be considered in isolation but a global approach to the problem of antimicrobial resistance is needed.
- Biosecurity (i.e. measures taken to keep diseases out of populations, herds, or groups of animals where they do not currently exist or to limit the spread of disease within the herd) should be promoted.
- Veterinarians and farmers should be continuously educated on strategies to minimise antimicrobial resistance
- Emergence of cephalosporin resistance in pathogenic and indicator bacteria should be monitored and the need for interventions should be continuously evaluated.
- Use of cephalosporins should be monitored in each country and this should be done by animal species to measure the effect of interventions described above. Data should be reported so that topical and systemic use is separated, and use of higher generations of cephalosporins can be distinguished.
- All Member States should implement and enforce internationally recognised codes of practice of rational and prudent use of antimicrobials (Codex code of practice to minimize and contain antimicrobial resistance CAC/RCP 61-2005; the OIE terrestrial code – chapter on antimicrobial resistance)
- Effect of chosen strategies should be monitored where possible in order to intervene if other strategies are necessary.
- Advertisement of cephalosporins should not be directed to animal owners

Recommendations on meticillin resistant *Staphylococcus aureus* (MRSA) in livestock, companion animals and food[23]

- It is recommended that periodic monitoring of intensively reared animals is carried out. This would provide trends in the development of this epidemic in all Member States. Data that would be comparable with the ongoing on-farm base-line study in breeding pigs would be useful in countries where the problem already exists, and may be extended to fattening pigs, veal calves and poultry. The preferred sampling method would be the collection of dust samples. In countries with a low or zero prevalence, studies at the abattoir level may be sufficient to detect the emergence of LA-MRSA. Although the preferred sampling method at the abattoir level has not yet been established, nasal swabs of pigs and cattle should be considered.
- In order to identify trends in the spread and evolution of zoonotically acquired MRSA, systematic surveillance and monitoring of MRSA in humans and food producing animals is recommended in all Member States. Harmonised data, including information on risk factors, as well as analysis of a representative sample of isolates for susceptibility to multiple antimicrobial agents, virulence associated traits, and lineage determination, should be available from a single location.
- In order to evaluate the effectiveness of control measures to reduce the carriage of CC398 in livestock, intervention studies should be carried out. Such studies should be longitudinal over consecutive production cycles.
- Further work should be performed on harmonising methods for sampling, detection and quantification of MRSA during carriage in both humans and animals, as well as for detection of MRSA as a contaminant of food, and in the environment including from dust both in air and on surfaces.
- The factors responsible for host specificity, persistence in different environments, transmission routes (including airborne transmission) and vectors, should be investigated.
- In order to evaluate the effectiveness of control measures to reduce the carriage of MRSA in companion animals and horses and their human contacts, intervention studies should be carried out.
- On the base of already existing recommendations for prevention of MRSA infections in some MSs, protocols for screening at admission to hospitals should be expanded to include humans exposed to intensively reared livestock.
- Due to the multiresistant character of MRSA, there are several antimicrobial classes that may increase the risk of spread of MRSA. Therefore, to be effective to control the emergence of MRSA, measures to reduce the use of antimicrobials cannot be limited to any specific class but routine use of antimicrobials is to be regarded as a risk factor. Any measures to be taken should consider all antimicrobials with the aim to

23 Joint scientific report of ECDC, EFSA and EMEA on meticillin resistant Staphylococcus aureus (MRSA) in livestock, companion animals and foods. EFSA-Q-2009-00612 (EFSA Scientific Report (2009) 301, 1-10) and EMEA/CVMP/SAGAM/62464/2009.

eliminate unnecessary use or replace use with other strategies. Thus adherence to the principles of prudent use remains a key measure to manage risks for spread of MRSA as discussed in the CVMP strategy on antimicrobials 2006-2010 and status report on activities on antimicrobials (EMEA/CVMP/353297/2005[24]) remains crucial. Special consideration should be given to improving controls related to group and flock medication of food producing animals and routine perioperative treatment of companion animals and horses when implementing these guidelines.

- Development of non-antimicrobial control measures should be encouraged. Further studies are required to document the long-term carriage of MRSA, and to find effective ways to decolonize animals and to clear the organism from different animal husbandry settings. The clonal nature of the Livestock Associated MRSA (LA-MRSA) theoretically presents opportunities for vaccine development but further research would be required. Use of antimicrobials for decolonisation seems to be of limited value.
- Appropriate wound management without antimicrobials will be sufficient for many MRSA infections. If antimicrobial treatment is necessary, based on the severity of the infection, there is a need to manage the risk of emergence of further resistance in the strain of MRSA infecting the animals to avoid subsequent spread of resistance to animals and humans. Due to the multiresistant nature of MRSA it may be difficult to find approved veterinary medicinal products for the condition. Last resort human medicines for MRSA treatment such as e.g. glycopeptides, oxazolidones, tigecycline and streptogramins have no maximum residue limit (MRL) and therefore they are not allowed to be used in animals intended for food production (Council Regulation (EEC) No 2377/90). Any use of such molecules in companion animals and horses should take into account the public health risk involved and should therefore involve discussions with public health practitioners.
- Monitoring of the consumption of antimicrobials in the EU is needed to identify and target action towards sources of unnecessary use of antimicrobials. This will also allow for evaluation of the effectiveness of measures taken in this respect.

Recommendations on EFSA Opinion 'Foodborne antimicrobial resistance as a biological hazard'[25]

- The development and application of new approaches to the recognition and control of food as a vehicle for AMR bacteria and related genes based on epidemiological and source attribution studies directed towards fresh crop-based foods, raw poultry meat raw pigmeat and raw beef are recommended.
- The use of epidemiological cut-off values provides an appropriate level of sensitivity when measuring resistance development in bacteria. These criteria have been harmonised for use in both in human and veterinary medicine in the European Union. It is now important that these matters be addressed globally.
- Specific measures to counter the current and developing resistance of known pathogenic bacteria to fluoroquinolones as well as to 3rd and 4th generation cephalosporins found in a variety of foods and in animals in primary production now require to be defined and put in place as a matter of priority.
- As a major source of human exposure to fluoroquinolone resistance via food appears to be poultry, whereas for cephalosporin resistance it is poultry, pork and beef that are important, these food production systems require particular attention to prevent spread of such resistance from these sources.
- If a full risk assessment for a specific food-bacterium combination, in respect of AMR, should be undertaken, methodologies currently available for the risk assessment of foods require to be modified for uniform adaptation at both MS and EU level for the risk assessment of those combinations (including foods originating from food animals, fish, fresh produce (e.g. lettuce etc.) and water, as a vehicle for the transmission of AMR bacteria and related genes).
- Further research on the role of commensals and of bacteria intentionally added as an aid to food processing in the transmission of AMR via food to the human flora, aimed at identifying ways in which such transmission from these agents can be prevented, is recommended.

24 http://www.emea.europa.eu/pdfs/vet/swp/35329705.pdf
25 Scientific Opinion of the Panel on Biological Hazards on a request from the European Food Safety Authority on foodborne antimicrobial resistance as a biological hazard. The EFSA Journal (2008) 765, 1-87.
http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/biohaz_op_ej765_antimicrobial_resistance_en,3.pdf?ssbinary=true.

- The role of food, water and the environment in the spread of apparently epidemic plasmids encoding multiple resistance is not clear, but deserves immediate attention.

- Overall, control of all the routes by which AMR bacteria and their related genes can arise in the human patient, of which food is but one such route, requires a response from all stakeholders to acknowledge their responsibilities for preventing both the development and spread of AMR, each in their own area of activity including medicine, veterinary medicine, primary food animal production, food processing and food preparation, as well as in the regulation of food safety.

Recommendations from the SCENIHR opinion on Biocides[26]

- Prudent use guidelines for biocides in their various applications should be evaluated and harmonized. In addition, surveillance programmes investigating bacterial resistance to biocides are recommended.

- There are currently no clear and well-referenced criteria or standards for the evaluation of the capability of a biocide to induce/select for antibiotic resistance. Therefore, tools need to be developed to define the "minimal selecting concentration": the minimal concentration of a biocide which is able to select or trigger the emergence/expression of a resistance mechanism concerning an antibiotic class in a defined bacterium.

- It should be noted that biocidal products are complex formulations (including various active ingredients) which potentiate the activity of individual active ingredients. It is important to take into account the evolution of the European regulation: n°1451/2007 (4th December 2007) and the recent European decision (2008/809/CE – 14th October 2008) with the suppression of numerous active substances. The impact of this decision on decreasing the overall activity of a formulation should be considered in future risk assessments.

- Considering the high uncertainty in the in vivo evaluation of the effects of biocides on the emergence of antibiotic resistance, reporting of production and use of biocides should be promoted.

- Environmental monitoring programmes for undesirable substances should include biocides.

26 SCENIHR, 2009. Scientific Committee on Emerging and Newly Identified Health Risks Opinion on: Assessment of the Antibiotic Resistance Effects of Biocides. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf.

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APPENDIX A

Examples of diseases on the different species including organ, pathogen, incidence and if is treated individually or as a flock treatment

	Organ	Disease	Incidence of the disease	Pathogen	Treatment type[27]
Cattle					
Dairy cows	Udder	Mastitis	Frequent	*Staphylococcus aureus* Coagulase negative staphylococci *Streptococcus uberis* *Streptococcus agalactiae* *Streptococcus dysgalactiae* *Escherichia coli* *Klebsiella* spp. *Arcanobacterium pyogenes*	Indivi-dual/ group for prevention
	Reproduc-tion system	Metritis/ Retained placenta and fetal membranes	Frequent	*Arcanobacterium pyogenes* *E. coli* *Staphylococcus* spp. *Streptococcus* spp.	Individual
	Joint/Digits	Joint infections	Frequent	*Arcanobacterium pyogenes*	Individual
		Footrot, digital dermatitis	Frequent	*Bacteroides* spp. *Fusobacterium* spp. *Pseudomonas* spp. *Staphylococcus* spp. Spirochetes	
	Intestine	Salmonellosis	Frequent	*Salmonella* spp.	Individual
Calves	Intestine	Enteritis	Frequent	*E. coli* F5 (ETEC) *Salmonella spp.*	Flock
	Blood	Septicaemia	Frequent	*E. coli* O15, O78 *Salmonella* spp. *Staphylococcus* spp. *Streptococcus* spp.	Individual
	Skin	Umbilical infections and polyserositis	Frequent	Mixed infections *E. coli*	Individual
	Lung	Pneumonia	Frequent	*Pasteurella spp* *Mannheimnia haemolytica* *Histophilus somnus* *Pasteurella multocida* *Arcanobacterium pyogenes*	Indivi-dual/ Flock
	Blood	Calf diphtheria		*Fusibacterium necrophorum* *Arcanobacterium pyogenes*	
	Digits	Footrot		*Arcanobacterium pyogenes* *Bacteroides* spp. *Fusobacterium* spp. *Pseudomonas* spp. *Staphylococcus* spp.	Individual
Pigs					
Sows	Skin	Erysipelas		*Erysipelothrix rhusiopathiae*	Individual
	Joints	Joint infections	Frequent	Haemolytic *Streptococcus* spp. *Streptococcus suis* *Staphylococcus aureus*	Individual

27 *Treatment here only refers to antimicrobial therapy. For many diseases, the primary treatment approach or prevention does not rely on antimicrobial agents (E. g. mastitis).

	Organ	Disease	Incidence of the disease	Pathogen	Treatment type[27]
				Erysipelothrix rhusiopathiae *Mycoplasma hyosynoviae*	
	Digits	Foot rot		Various agens	Individual
	Udder	Mastitis	Frequent	*Staphylococcus aureus* *Arcanobacterium pyogenes* *E. coli* *Klebsiella pneumoniae* Haemolytic *Streptococcus* spp *Staphylococcus aureus*	Individual
	Reproduc-tion system	Metritis	Frequent	Mixed infections	
		Mastitis/Metritis/Agalactiae	Frequent	*E. coli*, *Streptococcus* spp. *Staphylococcus* spp.	Individual/ Flock
Wea-ners	Intestine	Enteritis	Frequent	*E. coli*	Flock
	Blood	Septicemia		Haemolytic *Streptococcus* spp. *Streptococcus suis* *Escherichia coli* *Haemophilus parasuis (Glässer)*	Individual/ Flock
	Brain	Meningitis	Frequent	*Streptococcus suis*	Flock
	Skin	Umbilical infections	Frequent	Mixed infections	Individual
		Exudative epidermitis	Frequent	*Staphylococcus hyicus*	Flock
Gro-wing pigs	Intestine	Enteritis	Frequent	*Lawsonia intracellularis* *Brachyspira hyodysenteriae* *Brachyspira pilosicoli* *Clostridium perfringens* *Escherichia coli*	Flock
	Lung	Pneumonia	Frequent	*Actinobacillus pleuropneumoniae* *Pasteurella multocida* *Streptococcus suis* *Mycoplasma hyopneumoniae* *Mycoplasma hyorhinis*	Flock
		Whooping cough		*Bordetella bronchiseptica*	
	Skin	Tail bite infections		*Arcanobacterium pyogenes* *Staphylococcus aureus*	Individual/ Flock
Poultry					
Chi-ckens	Intestine	Enteritis	Frequent	*Clostridium* spp. *Enterococcus* spp. *E. coli*	Flock
	Respiratory system	Sinusitis	Frequent	*Bordetella* spp. *Avibacterium* spp. *Riemerella anatipestifer* *Clostridium* spp. *Mycoplasma* spp.	Flock
		Lung	Frequent	*Ornithobacterium rhinotracheale*	Flock
	Blood	Septicaemia	Frequent	*E. coli* *Erysipelothrix rhusiopathiae* *Pasteurella multocida* *Streptococcus* spp.	Flock
	Reproduc-tion system	Yolk Sac		*Pseudomonas aeruginosa*	Flock