



**COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE  
(CVMP)**

**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**

<b>DRAFT AGREED BY SAGAM (SCIENTIFIC ADVISORY GROUP ON ANTIMICROBIALS)</b>	18 October 2007
<b>ADOPTION BY CVMP FOR RELEASE FOR CONSULTATION</b>	13 February 2008
<b>END OF CONSULTATION (DEADLINE FOR COMMENTS)</b>	31 July 2008
<b>SCIENTIFIC CONTENT AGREED BY SAGAM</b>	27 November 2008
<b>ADOPTION BY CVMP OF SCIENTIFIC CONTENT</b>	12 February 2009
<b>ADOPTION BY CVMP OF RECOMMENDATIONS FOR ACTION</b>	11 March 2009

\* The revised document includes the CVMP recommendations for action

## RECOMMENDATIONS FOR ACTION

The CVMP adopted the review of its Scientific Advisory Group on Antimicrobials (SAGAM) on cephalosporins, which forms the body of this reflection paper, and discussed the need for measures to be taken with regard to the veterinary use of this group of substances. Available data indicate that resistance to 3rd generation cephalosporins, although still at low incidence in most EU countries, is increasingly reported in *E. coli* and *Salmonella* from animals in Europe.

Bacteria in livestock resistant to 3rd and 4th generation cephalosporins are considered as a food safety hazard as they might be human pathogens (e.g. *Salmonella*), or contribute to horizontal spread of resistance genes. Furthermore, cephalosporins are listed as critically important antimicrobials for both human and veterinary use (Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials, Rome 2007) and they are widely used in human medicine. The incidence of resistance to 3rd generation cephalosporins in, e.g. *K. pneumoniae* and *E. coli* in human infections is increasing in Europe. Many of these problems in human medicine can be correlated to use of cephalosporins and other antimicrobials in humans, but it is possible that spread from animal reservoirs via food or via the environment contributes to the dissemination of resistance in the community.

Data on the extent of use of 3rd and 4th generation cephalosporins for animals in the EU are not available in a way that allows exposure to be properly assessed. Cephalosporins for systemic use are mainly parenterally administered in individual animals, only cephalexin is used by oral route in some EU countries. Off label use cannot be quantified but has been reported.

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, CVMP considers it wise to take action on the veterinary side to reduce the possible risk for veterinary use contributing to emergence of resistance in human pathogens. Furthermore, action is needed in order to maintain the efficacy of cephalosporin-containing veterinary medicinal products. In general, prudent use of antimicrobials should be strongly promoted, and the cephalosporin group is one of the groups of antimicrobials which cause specific concern due to its importance in both human and veterinary medicine.

For veterinary medicinal products for food producing animals the CVMP recommends:

In the SPC for all products containing 3rd and 4th generation cephalosporins the following should be reflected:

- For systemically administered broad spectrum cephalosporins (3rd and 4th generation) it should be reflected 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 <antimicrobial>. Official, national and regional antimicrobial policies should be taken into account when the product is used.

The following recommendations are for consideration by Competent Authorities:

- 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.

In order to achieve a harmonised situation in SPCs of cephalosporin containing products in the EU, there is a need for harmonisation of prudent use instructions in the product literature of those products. The goal is already set out in the CVMP revised SPC guideline for antimicrobial products (EMA/CVMP/SAGAM/383441/2005). The harmonisation of prudent use instructions in the product literature could be achieved voluntary with the agreement of the veterinary pharmaceutical industry; otherwise regulatory actions would have to be put in place by regulators.

Notwithstanding the list of recommendations above, the CVMP is of the opinion that cephalosporins should not be considered in isolation but a global approach to the problem of antimicrobial resistance is needed. Therefore, the CVMP, in addition to the recommendations above, strongly supports the following more general suggestions to reduce antimicrobial resistance. The list is limited to actions related to veterinary medicine and includes (but is not limited to) specific recommendations for cephalosporins. It is recognised that those suggestions are outside the remit of the CVMP.

<b>Suggested action</b>	<b>Responsible body</b>
<ul style="list-style-type: none"> <li>• 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.</li> </ul>	Farmer's organisations, competent authorities and related stakeholders.
<ul style="list-style-type: none"> <li>• Veterinarians and farmers should be continuously educated on strategies to minimise antimicrobial resistance</li> </ul>	Universities, Veterinary and Farmers Associations, National Authorities (e.g. granting veterinary authorisation)
<ul style="list-style-type: none"> <li>• Emergence of cephalosporin resistance in pathogenic and indicator bacteria should be monitored and the need for interventions should be continuously evaluated.</li> </ul>	The European Commission, EFSA, ECDC, Community Reference Laboratory, National Reference Laboratories and routine laboratories
<ul style="list-style-type: none"> <li>• 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.</li> </ul>	Member State Competent Authorities
<ul style="list-style-type: none"> <li>• 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)</li> </ul>	Member States
<ul style="list-style-type: none"> <li>• Effect of chosen strategies should be monitored where possible in order to intervene if other strategies are necessary.</li> </ul>	Member States
<ul style="list-style-type: none"> <li>• Advertisement of cephalosporins should not be directed to animal owners</li> </ul>	Member States

## TABLE OF CONTENTS

RECOMMENDATIONS FOR ACTION .....	2
TABLE OF CONTENTS .....	4
MANDATE .....	5
INTRODUCTION.....	5
OBJECTIVE.....	5
BACKGROUND.....	6
Mechanism of action, classification and spectrum of activity .....	6
Use of cephalosporins in human medicine.....	6
CEPHALOSPORINS FOR FOOD PRODUCING ANIMALS.....	7
Cephalosporins authorised for animals in the EU.....	7
Use of cephalosporins for animals in the EU.....	9
RESISTANCE MECHANISMS AND GENETICS .....	11
Resistance to cephalosporins in staphylococci.....	11
Resistance to cephalosporins in <i>Enterobacteriaceae</i> .....	11
RESISTANCE IN BACTERIA FROM FOOD PRODUCING ANIMALS.....	14
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA).....	14
Resistance in <i>Enterobacteriaceae</i> .....	14
Emergence of ESBLs .....	16
Emergence of transferable AmpC-type beta-lactamases .....	18
INFLUENCE OF USE OF ANTIMICROBIALS ON THE EMERGENCE AND SPREAD OF RESISTANCE.....	19
Influence of cephalosporin use on occurrence of MRSA .....	19
Influence of cephalosporin use on the evolution of genes encoding beta-lactamases .....	19
Influence of cephalosporin use on selection and amplification of genes encoding beta- lactamases.....	19
Co-selection of resistance in <i>Enterobacteriaceae</i> by non-cephalosporin antimicrobials .....	21
EXPOSURE OF HUMANS TO RESISTANT BACTERIA AND RESISTANCE GENES FROM ANIMAL SOURCES.....	21
Exposure to resistant bacteria from animals .....	21
Exposure to resistance genes from bacteria associated with food of animal origin.....	22
IMPACT OF INFECTIONS WITH CEPHALOSPORIN-RESISTANT BACTERIA ON HUMAN AND ANIMAL HEALTH .....	23
Human health .....	23
Animal health .....	24
SUMMARY ASSESSMENT.....	25
REFERENCES.....	27

## MANDATE

The Scientific Advisory Group on Antimicrobials (SAGAM) was mandated to give advice to the CVMP on the need to exercise certain control on those classes of compounds of greater importance to human medicine e.g. fluoroquinolones and 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins.

This document discusses cephalosporins with a focus on substances of the 3<sup>rd</sup> and 4<sup>th</sup> generation and food producing animals, excluding aquaculture.

## INTRODUCTION

Cephalosporins of the 3<sup>rd</sup> and 4<sup>th</sup> generation represent subclasses of antimicrobials very important in the treatment of severe and invasive infections in humans; such antimicrobials are therefore of special interest from a public health perspective. The first nosocomial outbreaks of bacteria resisting these cephalosporins by production of beta-lactamases were described in the 1980s (Gniadkowski, 2001). Subsequently the occurrence of infections with bacteria resistant to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins e.g. *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp and *Pseudomonas aeruginosa* has increased worldwide. Increased resistance implies either delayed adequate treatment or initial use of second and third line alternatives. Some of the latter carry a higher risk of adverse reactions (e.g. aminoglycosides) or are clearly more toxic (e.g. colistin). Due to delayed adequate therapy, the burden of infections with bacteria resistant to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins can be substantial with severe outcomes, including both higher overall and higher infection-related mortality, increased length of hospital stay and higher costs (Schwaber & Carmeli, 2007).

In Europe infections with bacteria resistant to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins were previously mainly caused by *Klebsiella pneumoniae*, and were mostly diagnosed in specialist units (Livermore *et al.*, 2007). In the last decade this pattern has changed and resistance is rapidly emerging, not only in hospitals but also in community-acquired infections. Pathogens carrying genes encoding these resistance traits now include *E. coli* and *Salmonella* (Anonymous, 2006a; Canton & Coque, 2006; Livermore *et al.*, 2007). Linkage to other resistance genes and co-selection by unrelated antimicrobials are important in the epidemiology of these resistance genes (Canton & Coque, 2006).

This change in epidemiology, with typically nosocomial organisms spreading to and from the community, indicates an exchange of organisms or genes with other, perhaps non-human bacterial reservoirs. Resistance has emerged in some countries in both *Salmonella* and *E. coli* from food producing animals. This suggests that food of animal origin may be one of the potential vectors for dissemination of transferable beta-lactamase-encoding genes in the community (Livermore *et al.*, 2007, Carattoli 2008). Third and 4<sup>th</sup> generation cephalosporins are used for food producing animals and could potentially influence the prevalence of resistance. In addition, co-selection by other antimicrobials used for medication of large groups of animals (mass medication) may contribute to the occurrence and dissemination of resistance determinants in animals.

## OBJECTIVE

The objective of this document is to critically review recent information on the use of cephalosporins of the 3<sup>rd</sup> and 4<sup>th</sup> generation in food producing animals in the EU, its effect on development of resistance to this category of antimicrobial agents in bacterial species that are of importance for human and animal health, and the potential impact on human and animal health.

## BACKGROUND

### Mechanism of action, classification and spectrum of activity

The mechanism of antibacterial activity of the cephalosporins and cephamycins is essentially the same as for benzylpenicillin and other beta-lactam antimicrobials; they interfere with the formation of the cell wall by binding to enzymes that are active in the synthesis of peptidoglycans (transpeptidases, also called penicillin binding proteins, PBPs). All true cephalosporins contain a 7-aminocephalosporanic acid molecule, composed of a beta-lactam ring essential for activity, and a six-membered dihydrothiazine ring. A wide variety of cephalosporins has been generated by substitutions of various groups at different positions of the nucleus. The cephamycins differ from the true cephalosporins by the presence of a methoxy-group in the position 7 of the cephalosporin nucleus, and are stable to many beta-lactamases.

The cephalosporins and cephamycins are grouped together and are classified on basis of their *in vitro* spectrum of activity, structural similarities and to some extent, the year of introduction. In this document, the term cephalosporins will be used to cover both true cephalosporins and cephamycins, unless specifically indicated. The traditional classification of these molecules into 'generations' will be followed and they are grouped according to the Anatomic Therapeutic Chemical (ATC) index of January 2005 (Anonymous, 2005) and ATCvet:

- *First generation cephalosporins* (e.g. cephalexin, cefadroxil, cephalotin) have the narrowest spectrum of activity. They have an excellent activity against Gram-positive cocci, including penicillinase-producing staphylococci but the activity against Gram-negative bacteria is limited.
- *Second generation cephalosporins* (e.g. cephacloclor, cefoxitin<sup>†</sup>, cefuroxime) have an expanded spectrum of activity compared with first generation substances and are generally more active against Gram-negative bacteria.
- *Third generation cephalosporins* (e.g. cefotaxime, ceftiofur, cefoperazone, latamoxef<sup>®</sup>) generally have a broad spectrum of activity, with increased stability to many of the beta-lactamases that inactivate the earlier generation's cephalosporins and other beta-lactam antimicrobials.
- *Fourth generation cephalosporins* (e.g. cefepime, cefpirome, cefquinome) have an even more extended activity against Gram-negative bacteria, as they have a further increased stability compared with the third generation compounds.

### Use of cephalosporins in human medicine

Cephalosporins are widely used in human medicine, both in hospitals and in the community. In hospitals, 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins are used to treat, e.g. septicaemia, meningitis, hospital acquired pneumonia, intra-abdominal infections and complicated urinary tract infections (Paterson & Bonomo, 2005). The total hospital consumption of antimicrobials in 15 European countries in 2002 ranged from 1.28 to 3.89 defined daily doses (DDD)/1000 inhabitants per day (Vander Stichele *et al.*, 2006). The proportion of cephalosporin use ranged from 8 to 31% of the total use, and within the group the proportion of 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins ranged between 10 and 50%. An increase in the hospital use of 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins between the years 1997 and 2002 was noted for all 15 countries.

In 2003, the total outpatient use of antimicrobials (i.e. for non-hospitalised patients) in 34 European countries ranged from 9.78 to 31.40 DDD/1000 inhabitants per day (Ferech *et al.*, 2006). The cephalosporin use in 25 of these countries ranged 0.02 to 6.18 DDD/1000 inhabitants per day, which equates a factor of 270 between the highest and lowest using country (Coenen *et al.*, 2006). In many countries, most or almost all of this use was 1<sup>st</sup> and 2<sup>nd</sup> generation cephalosporins. Cephalosporins of higher generations have mainly been available for injection or infusion, and their use has probably

---

<sup>†</sup> a cephamycin

mostly been limited to patients in e.g. elderly care with severe community acquired pneumonia or complicated urinary tract infections. Some products for oral use have been introduced on the market. In three countries, use of 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins was more than 40% of the total outpatient use of cephalosporins in 2003, compared to others with almost no such use at all. This extreme variation is probably explained by inappropriate use of 3<sup>rd</sup> generation cephalosporins for uncomplicated urinary and respiratory tract infections in some countries (Coenen *et al.*, 2006).

The total hospital consumption of antimicrobials has been estimated to be about 5-10% of the total consumption (Vander Stichele *et al.*, 2006). Hospital exposure is more concentrated in terms of number of patients in the population exposed and the intensity of treatment. This provides a selective pressure that if combined with inadequate infection control and with an inherent accumulation of vulnerable patients, creates conditions for emergence and spread of infections with resistant bacteria within the hospital and eventually to the community.

## CEPHALOSPORINS FOR FOOD PRODUCING ANIMALS

### **Cephalosporins authorised for animals in the EU**

Cephalosporins have been authorised for use in food producing animals via national procedure, mutual recognition or centralised procedure. One product with cephalosporins (ceftiofur) is authorised centrally. The number of products containing the various active ingredients for some EU countries plus Iceland, authorised for food producing animals by national procedure or by mutual recognition procedure, is illustrated in Figure 1 (systemic use) and 2 (intramammary use). In addition, cephalixin is authorised for use in water or milk-replacers in at least two Member States and cefapirin and cefquinome in several Member states for intrauterine use.

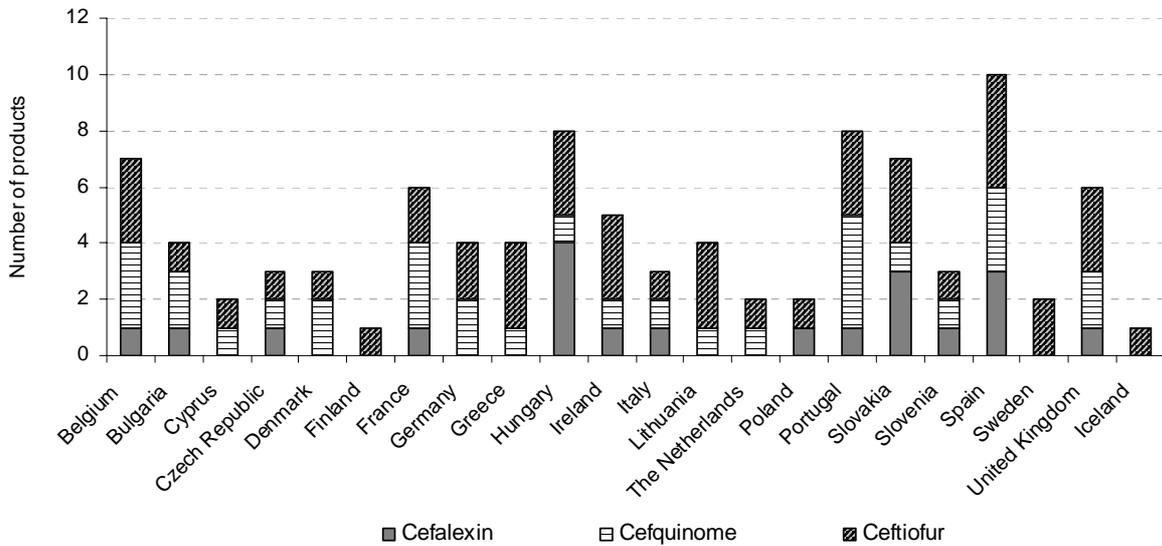


Figure 1. Number of products formulated for injection per antimicrobial substance and Member State (data from 2006).

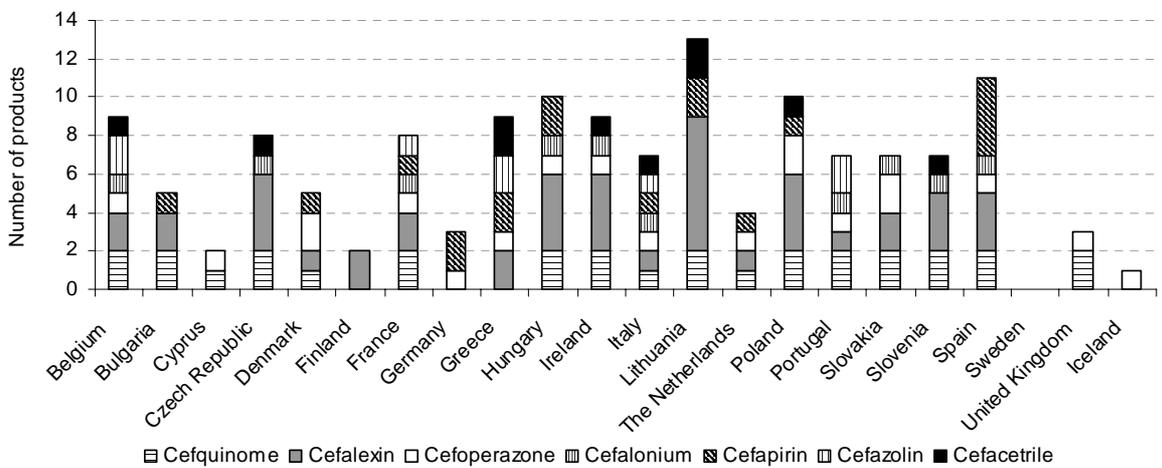


Figure 2. Number of products formulated for intramammary use per antimicrobial substance and Member State (data from 2006).

Maximum residue limits (MRLs) have been established for cattle for all substances shown in Figure 1 and 2, and in addition for sheep and goat for cefazolin, for pig for ceftiofur (the current entry for this substance in the Annexes of Council Regulation (EEC) No 2377/90 includes all mammalian food producing species) and for pigs and horses for cefquinome. Presently, no MRLs have been established for poultry.

Regarding potential effects of use of cephalosporins on resistance in bacteria, systemic use particularly for groups or flocks of animals, is likely to have the major impact. This is because microbiota in multiple body sites are exposed. This document will therefore focus on potential effects of systemic use. Of the substances authorised for systemic use, cephalixin is a 1<sup>st</sup>, ceftiofur a 3<sup>rd</sup> and cefquinome a 4<sup>th</sup> generation cephalosporin.

Intramammary use (i.e. local use) leads to a lesser exposure of the normal microbiota of the target animal. It is recognised that cephalosporins are widely used as lactating or dry cow therapy in many Member States (Figure 2). Such use may have an impact on selection of resistance in target bacteria. Furthermore, if milk from recently treated cows is fed to calves before the withdrawal time has elapsed, the intestinal microbiota of the latter may be exposed. In some countries, these practises might be widespread (Sampion *et al* 2008).

Ceftiofur (free acid) is centrally authorised for subcutaneous administration in pigs with an extended dosage interval ('long acting') for treatment of respiratory tract infections, septicaemia and polyarthritis and polyserositis caused by defined pathogens. Ceftiofur hydrochloride (not 'long acting') is authorised in most countries for intramuscular administration in cattle and pigs with indications for treatment of respiratory disease, and in cattle also for interdigital necrobacillosis and puerperal metritis. In some Member States, ceftiofur was previously authorised for injection of day-old chickens for prevention of septicaemia (Bertrand *et al.*, 2006). There are currently no cephalosporin-containing products authorised for poultry species in the EU.

Cefquinome (4<sup>th</sup> generation) is available in some Member States for systemic use in cattle, pigs and horses. The indications for use are mainly respiratory infections, interdigital necrobacillosis in cattle, septicaemia caused by *E. coli* in calves and foals, and streptococcal infections in horses. In some countries, indications such as bovine mastitis caused by *E. coli*, post-partum dysgalactia syndrome in sows (previously "MMA" syndrome) and meningitis and arthritis in piglets are also included. For ceftiofur, formulations of cefquinome for subcutaneous administration with extended dosage intervals are authorised in some Member States.

## **Use of cephalosporins for animals in the EU**

Information on the consumption of antimicrobial agents for food producing animals is not readily available for most Member States, although the availability of such data is slowly improving. Reported data are mostly compiled for all animal species, including dogs and cats. Further, data are often reported as "beta-lactam antimicrobials", i.e. including also benzylpenicillin and phenoximethylpenicillin, ampicillin etc. Finally, in the few reports where information on penicillins and cephalosporins are given separately, data are not further divided into generations. It is therefore not possible to compile comparable and relevant data on the use of cephalosporins of different generations in the Member States.

As an example of amounts used, available data on use of all cephalosporins in some Member States are presented in Table 1. Although these figures cannot be regarded as representative for all Member States, some observations can be made. A substantial part (55% to 98%) of all use of cephalosporins in these countries is for pets. Until recently only first generation-cephalosporins have been authorised for pets. By contrast, the systemic use for food producing animals is likely to be dominated by 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins. In the reports on use of antimicrobials from Denmark and France, data are given per animal species. The amounts for systemic use for pigs were 98 kg and 1310 kg active substance respectively, representing 51% and 89% of the total use for food producing animals.

Table 1. Sales of cephalosporins (all generations) for systemic use in food producing animals and pets, and for intramammary use in Denmark, Finland, France and Sweden, expressed as kg active substance (years 2005 or 2006)<sup>1</sup>.

	Systemic use:		Intramammary use	Total
	Food producing animals	Pets		
Denmark	193	354	91	638
Finland	0.2	915	85	1000
France	1480	5420	1610	8510
Sweden	26	1186	0.1	1212

<sup>1</sup>Source: AFSSA 2005, DANMAP 2006, FINRES-vet 2005-2006, SVARM 2006

Ceftiofur and cefquinome are both mainly administered parenterally. It has been argued that this would limit their use to special situations. Other factors such as the very broad spectrum, short or zero withdrawal times for milk and the availability of ‘long acting’ formulations for certain indications are factors which could also make these drugs a convenient choice in many situations.

Currently there is no harmonised approach on prudent use of cephalosporins in animals in different Member States. In some marketing authorisations in the EU, special precautions for use have been added to the Summary of Product Characteristics (SPCs) of cephalosporin products. The guideline of the Federation of Veterinarians of Europe on prudent use is at a general level and states: "where an appropriate narrow spectrum agent is available, it should be selected in preference to a broad spectrum agent" (FVE, 1999). Guidance on prudent use of antimicrobials for animals have been published in many countries (Passantino, 2007) but most are on a general level and cephalosporins are not specifically mentioned.

Some national guidelines give specific recommendations for the use of cephalosporins. For example, in the Dutch guidelines for therapy (Formularia) issued by the Royal Veterinary Association (KNMvD), drugs of eminent importance to public health are considered third choice drugs for treatment of infections in food animals (KNMvD, 2007). Third choice means: only to be used if no alternative therapy is possible, based on susceptibility test of the target pathogens. For individual animals with severely invasive infections, third choice drugs may be used for empiric first choice therapy. According to the German guidelines for prudent use of antimicrobials in veterinary medicine issued by the Federal Veterinary Association and the Working Group of Chief Veterinary Officers (2000), it is mandatory that reserve antimicrobials with last resort character in human medicine are used restrictively in individual animals on a short-term basis and only in cases where they are strictly indicated. The Finnish guidelines are even more specific; recommendations are given for specific indications in different animal species. Third-generation cephalosporins are advised, with specific cautions, only for treatment of foal septicaemia (Anonymous, 2003). National legislation in Finland prohibits the use of 3rd and 4th generation cephalosporins for animals unless a veterinary medicinal product containing these substances has a marketing authorization or a special licence. Off-label use of these products is prohibited.

Even when specific guidelines exist their implementation is generally not monitored. In specific as well as in general guidelines, off-label use is restricted to situations where no other suitable product is available and should be carefully justified (Passantino, 2007). Despite these recommendations off-label use for non-authorized indications can be common. For example in ten pig farms in Denmark, off-label use of ceftiofur was common (Jorgensen *et al.*, 2007). The authorised indications for the use of ceftiofur in pigs is treatment of respiratory tract infections, septicaemia, polyserositis and polyarthritis, but eight farms used the drug for systemic prophylaxis in newborn piglets and one for treatment of diarrhoea. Information on indications authorised in countries outside EU, such as injection *in ovo* or of day-old poultry, or claimed advantages such as routine prophylactic use of ceftiofur for weaning pigs is easily available on the internet and this might sometimes influence the veterinarians’ choice of therapy.

## RESISTANCE MECHANISMS AND GENETICS

### **Resistance to cephalosporins in staphylococci**

In staphylococci, resistance to penicillinase sensitive penicillins (benzylpenicillin, phenoximethylpenicillin and aminopenicillins) is caused by narrow spectrum beta-lactamases (Li *et al.*, 2007). Resistance to all beta-lactams, including the cephalosporins, is caused by the alteration of the penicillin-binding proteins (PBPs). The altered PBP has a low affinity for beta-lactam antimicrobials. This mechanism is generally referred to as methicillin<sup>‡</sup> resistance (Li *et al.*, 2007). The gene encoding this mechanism, *mecA*, is chromosomally-located as part of the Staphylococcal Cassette Chromosome (SCCmec). SCCmec is horizontally transferable between staphylococci and is commonly present in some species of coagulase negative *Staphylococcus* spp. present in humans and in animals.

### **Resistance to cephalosporins in *Enterobacteriaceae***

Resistance to cephalosporins in *Enterobacteriaceae* (eg. *Salmonella*, *E. coli*) is primarily caused by production of beta-lactamases with broad or extended spectrum, e.g. with substrate specificity not only for the penicillins but also for cephalosporins. To date, several hundred variants of beta-lactamases have been distinguished. The enzymes are classified according to different schemes. The most commonly used are those by Bush *et al.* (Bush *et al.*, 1995) based on functional properties, the Ambler system based on structural similarities (Ambler, 1980) or a combination of both (Bush *et al.*, 1995). For the purpose of this document a simplified overview of beta-lactamases is provided based on bacterial host and functional differences (Table 2). Unfortunately some of the enzymes have been given more than one name. A list of common homonyms and a comprehensive list of the origin of the names has recently been published (Jacoby, 2006) (for a complete list see <http://www.lahey.org/studies>).

---

<sup>‡</sup> According to international non-proprietary names (INN), the spelling is “meticillin”

Table 2. Main types of beta-lactamases among staphylococci, *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* (adapted from Jacoby & Munoz-Price, 2005)

Bacterial species	Beta-lactamase	Examples	Substrate specificity/Activity pattern	Susceptible to clavulanic acid <sup>a</sup>
<i>Staphylococcus aureus</i>	Penicillinase	PC-1	Benzympenicillin, aminopenicillins	++++
<i>Enterobacteriaceae</i> , <i>Pseudomonas</i> and <i>Acinetobacter</i>	Broad-spectrum	TEM-1, TEM-2, SHV-1	Benzympenicillin, aminopenicillin, ureidopenicillins, carboxypenicillins, 1 <sup>st</sup> generation cephalosporins (e.g. cephalothin).	+++
		OXA family (some)	As for the broad spectrum group plus penicillinase stable penicillins such as oxacillin	+
	Extended Spectrum	TEM family and SHV-family	Substrates of the broad-spectrum group plus oxiamino-cephalosporins (cefotaxime, ceftazidime, ceftiofur) and monobactams	++++
		Others (BES, GES/IBC family, PER, VEB, TLA, SFO)	As for TEM-family and SHV-family	++++
		CTX-M family	Substrates of the expanded-spectrum group plus, for some enzymes, cefepime	++++
		OXA-family (some)	Same as for CTX-M family	+
	AmpC	ACC, ACT, CMY, DHA, FOX-family, LAT-family, MIR, MOX	Substrates of the extended spectrum group except 4 <sup>th</sup> generation cephalosporins, but in addition cephamycins (e.g. cefotetan, ceftiofur)	0
	Carbapenemase	IMP-family, VIM-family, GIM and SPM	Substrates of the extended-spectrum group plus cephamycins and carbapenems	0
		KPC	Same as for IMP-family, VIM-family, GIM and SPM	+++
		OXA -23, -24, -25, -26, -27, -40, -48	Same as for IMP-family, VIM-family, GIM and SPM	+

<sup>a</sup> The number of plus-signs denote relative susceptibility of the families to inhibitors. Note that within the generally susceptible ESBL families, inhibitor resistant variants occur.

### *Extended spectrum beta-lactamases (ESBLs)*

The ESBLs often evolve from enzymes with a narrower spectrum such as the wide-spread TEM-1 and SHV-1. Amino acid substitutions or insertions by mutations in the genes encoding these enzymes lead to extended substrate specificity or increased hydrolytic rate (Gniadkowski, 2001; Jacoby & Munoz-Price, 2005). The number of known variants of the TEM and SHV families is constantly growing, and most have emerged (and are emerging) by stepwise mutations. The selection of a particular variant in a given hospital has often been related to a specific profile of use (Gniadkowski, 2001). In the last decade, plasmid mediated CTX-M enzymes (hydrolysing cefotaxime) have emerged and spread rapidly in *Enterobacteriaceae* in many parts of the world, including Europe (Canton & Coque, 2006). The CTX-M family can be sub-divided in several clusters and, as for the TEM- and SHV-families, mutational events lead to emergence of variants within each cluster (Livermore *et al.*, 2007).

The ESBL-encoding genes are often associated with genetic structures that are highly mobile. These may be large self-transmissible plasmids and/or transposons and integrons. Mobility and expression is further promoted by the association of many of the CTX-M genes with insertion sequences (Canton & Coque, 2006; Jacoby, 2006). The insertion sequences are probably responsible for their mobilisation from progenitors and can contribute to further dissemination. Integrons are genetic elements that are able to capture individual antimicrobial resistance gene cassettes. The integrons carrying genes encoding CTX-M-type enzymes are mostly of class 1 type, which are in turn associated with insertion sequences and often with transposons and plasmids (Canton & Coque, 2006).

### *Chromosomal and plasmid mediated AmpC type beta-lactamases*

The AmpC type beta-lactamases form a large group of originally species-specific enzymes encoded chromosomally in various Gram-negative bacteria. The amount of enzyme that is inherently produced varies between species depending on the mechanism of regulation. For example in *E. coli* the chromosomally encoded production of AmpC is normally repressed and the levels of the enzyme are then insufficient to confer ampicillin resistance. However, mutations in the promoter region can lead to derepression of the AmpC gene resulting in hyper-production of the enzyme, with clinical resistance to ampicillin and cephalosporins as a consequence (Batchelor *et al.*, 2005c; Gootz, 2004; Li *et al.*, 2007; Pfaller & Segreti, 2006).

Although enzymes of the AmpC type do not normally affect 4<sup>th</sup> generation cephalosporins, reports from human medicine indicate that on rare occasions, mutations in the genes encoding these enzymes can lead to an expanded spectrum AmpC (abbreviated ESAC) which includes resistance to 4<sup>th</sup> generation cephalosporins (Ahmed & Shimamoto 2008; Le Turnier *et al.*, 2008, Mammeri, H *et al.*, 2007; Mammeri *et al.* 2008; Wachino *et al.*, 2006).

In *Enterobacteriaceae* genes encoding AmpC-type beta-lactamases are increasingly associated with plasmids (Gootz, 2004). Apparently these genes have been mobilised from the chromosome of certain bacterial species in which they are inherent, evolved further and are now spread horizontally between different species of *Enterobacteriaceae* (Alvarez *et al.*, 2004; Biedenbach *et al.*, 2006; Gootz, 2004). For example, *Salmonella* does not inherently carry this type of enzymes but over the last decade, genes encoding variants of the enzyme CMY (cefamycinase) have been identified on plasmids in a large number of different *Salmonella* serovars (Arlet *et al.*, 2006). Plasmid encoded CMY production has also been identified in, e.g., *E. coli* of animal origin (Blanc *et al.*, 2006; Brinas *et al.*, 2005; Brinas *et al.*, 2003a; Brinas *et al.*, 2003b; Donaldson *et al.*, 2006; Kojima *et al.*, 2005).

### *Co-resistance*

The genes encoding ESBLs are often physically linked in integrons, transposons and/or plasmids with genes encoding resistance to other, structurally unrelated resistance genes (Canton & Coque, 2006). Co-resistance to e.g. aminoglycosides, tetracyclines and sulphonamides is frequent, not only in isolates from nosocomial outbreaks but also in isolates of *Salmonella* (Batchelor *et al.*, 2005b; Bertrand *et al.*, 2006; Hasman *et al.*, 2005; Li *et al.*, 2007; Michael *et al.*, 2006; Politi *et al.*, 2005; Weill *et al.*, 2004) and other *Enterobacteriaceae* from animals (Blanc *et al.*, 2006; Brinas *et al.*, 2003b; Kojima *et al.*,

2005). Multiresistant CTX-M-producing strains from humans have been shown to carry transferable quinolone resistance genes (*qnr* and/or *aac(6')-Ib-cr*) (Canton & Coque, 2006; Robicsek *et al.*, 2006a). The latter of these genes encodes the enzyme AAC(6')-Ib-cr, a variant of an aminoglycoside acetyltransferase that also modifies some fluoroquinolones via N-acetylation at the amino nitrogen on its piperazinyl substituent (Robicsek *et al.*, 2006b)

For CTX-M, genes encoding CMY and other plasmid mediated AmpC type resistance are frequently associated with other genes encoding resistance to structurally unrelated antimicrobials (Batchelor *et al.*, 2005c; Jacoby & Munoz-Price, 2005). Co-resistance with several other antimicrobials, e.g., aminoglycosides, chloramphenicol and florfenicol, sulphonamides, tetracycline and/or trimethoprim is common and has been documented in *Salmonella* and *E. coli* from animals and food (Alcaine *et al.*, 2005; Allen & Poppe, 2002; Berge *et al.*, 2004; Lopes *et al.*, 2006; White *et al.*, 2001; Zhao *et al.*, 2003) and *E. coli* from animals (Brinas *et al.*, 2003b; Donaldson *et al.*, 2006).

#### *Laboratory detection of ESBL and AmpC-type beta-lactamases*

Reliable laboratory detection of resistance mediated by ESBLs depends on screening for decreased susceptibility with several different cephalosporins. Use of both cefotaxime and ceftazidime or cefpodoxime and use of low break-points has been recommended for the testing of *Enterobacteriaceae* (Livermore & Brown, 2001). For surveillance purposes, epidemiological cut-off values such as those set by EUCAST are more sensitive in detecting organisms that harbour ESBLs or AmpCs, than the mostly higher clinical break-points (Kahlmeter, 2008). Recently EFSA advised that testing cefotaxime and use of epidemiological cut-off values should be sufficient to detect principally all ESBLs and AmpC type beta-lactamases (Anonymous, 2006b).

## RESISTANCE IN BACTERIA FROM FOOD PRODUCING ANIMALS

### **Methicillin-resistant *Staphylococcus aureus* (MRSA)**

Infections with MRSA in hospitals but also increasingly in the community are a major public health problem worldwide (Boyce *et al.*, 2005). Colonisation and infection with MRSA has been increasingly reported among pets and horses, and more recently in food producing animals (for a review see Leonard & Markey, 2008). In the Netherlands, high prevalence of a particular clone of MRSA, ST398, has been reported in pigs, but also in other animals (de Neeling *et al.*, 2007). The same clone has also been reported in animals in other European countries (Guardabassi *et al.*, 2007; Witte *et al.*, 2007), and from infections in humans in several countries. The “pig clone” was previously absent in human infections, which suggests that it has emerged in animals.

### **Resistance in *Enterobacteriaceae***

In Table 3 and 4, phenotypic data on resistance to 3<sup>rd</sup> generation cephalosporins in *E. coli* from healthy animals and in *Salmonella* have been compiled from the national zoonoses reports submitted to EFSA (available at [www.efsa.europa.eu](http://www.efsa.europa.eu)). Data from Enter-net (an EU-funded international surveillance network for the enteric infections *Salmonella* and VTEC O157; [http://www.hpa.org.uk/hpa/inter/enter-net\\_menu.htm](http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm)) indicate that in year 2005, the overall average figure of resistance to cefotaxime in *S. Typhimurium* isolated from humans in EU was 0.6% (Anonymous, 2006a). Comparability is hampered by differences in inclusion criteria, testing methodology and choice of interpretation criteria. In many cases (both in veterinary and human medicine), use of clinical break-points has probably lead to an underestimation of the occurrence of ESBLs (Kahlmeter, 2008).

Table 3. Reported resistance to cefotaxime or ceftiofur in *Escherichia coli* isolated in healthy animals in 2007 (number of investigated isolates and percent reported as resistant; based on data in national zoonoses reports submitted to EFSA in accordance with Directive 2003/99/EC, [www.efsa.europa.eu](http://www.efsa.europa.eu). Only entries with results from more than 10 isolates were included).

Country reporting	Method <sup>a</sup>	Cut-off <sup>b</sup> mg/l	Cattle		Fowl ( <i>Gallus gallus</i> )		Pigs	
			N <sup>e</sup>	% <sup>g</sup>	N	%	N	%
Austria	Dil	0.25	43	0	43	0	46	0
Denmark	Dil	1 <sup>c</sup>	98	0	114	2.0	150	0.7
Estonia	Dil	0.25	21	4.0			19	0
Finland	Dil	0.25					135	0
France	Dil	2	103	0	101	2.0	126	0.8
Italy	Dil	0.25			37	2.7	149	0.7
The Netherlands	Dil	0.25	152 <sup>f</sup>	0	43	9.0	169	1.2
Norway	Dil	0.25					198	1
Slovenia	DD	NA	22	0			34	2.9
Spain	Dil	0.25	158	0	87	24.1	229	0.9
Sweden	Dil	0.25			296	1.0		
Switzerland	Dil	4 <sup>c</sup>			284	0.4	98	0
United Kingdom	DD	NA	1652	6.7				

<sup>a</sup> Dil = (micro)dilution, DD= disk diffusion; <sup>b</sup> cut-off used to define resistance to cefotaxime unless otherwise indicated; <sup>c</sup> cut-off for ceftiofur; <sup>e</sup> Number of isolates tested; <sup>f</sup> Figure in table is for bovines unspecified. Also reports veal N=87, 1% resistance and dairy cattle N=18 5.6% resistance; <sup>g</sup> Percent of tested isolates reported as resistant;

Table 4. Reported resistance to cefotaxime or ceftiofur in *Salmonella* isolated from animals or food products in 2007 (number of investigated isolates and percent reported as resistant; based on data in national zoonoses reports submitted to EFSA in accordance with Directive 2003/99/EC, [www.efsa.europa.eu](http://www.efsa.europa.eu). Only entries with results from more than 10 isolates were included).

Country reporting	Method <sup>a</sup>	Cut-off <sup>b</sup> mg/l	<i>Salmonella</i> Enteritidis		<i>Salmonella</i> Typhimurium				<i>Salmonella</i> spp											
			Fowl ( <i>Gallus gallus</i> )		Cattle		Fowl ( <i>Gallus gallus</i> )		Pigs		Cattle		Gallus gallus (fowl)		Pigs		Meat from poultry		Meat from pigs	
			N <sup>e</sup>	% <sup>f</sup>	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Austria			78	0			13	0	14	0										
Czech republic	Dil	0.5	195	0					21	0										
Denmark	Dil	2 <sup>c</sup>			13	0			575	0							71	0		
Finland	Dil	0.5			14	0														
France	DD	NA	48	0	28	0	23	0	90	0										
Germany	Dil	4 <sup>c</sup>	97	0	112	0	22	0	276	0	304	0	208	0	484	0	214 <sup>f</sup>	0.9	117	0.9
Hungary																				
Ireland	Dil	0.5							41	0										
Italy	Dil	0.5	59	0			14	0	31	0	12	0					25	8.0	129	0
The Netherlands	Dil	0.5	19	0			11	0	84	0			134	16.4	43	0				
Slovakia	Dil	0.5	79	0							17	0	137	0	28	0	12 <sup>f</sup>	0		
Slovenia	DD	NA	13	0																
Spain	Dil	0.5	185	1.1					19	0										
Sweden	Dil	0.5					12	0	15	0					14	0				
United Kingdom	DD	NA	123	0	86	0			889	0					50	0				

<sup>a</sup> Dil = (micro)dilution, DD= disk diffusion; <sup>b</sup> cut-off used to define resistance to cefotaxime unless otherwise indicated; <sup>c</sup> cut-off for ceftiofur; <sup>e</sup> Number of isolates tested; <sup>g</sup> Percent of tested isolates reported as resistant; <sup>f</sup> isolates from broiler meat

As can be seen in Tables 3 and 4, some countries report a comparatively high prevalence of resistance to 3<sup>rd</sup> generation cephalosporins for *E. coli* from poultry and, for one country also cattle. Individual countries also report increasing resistance in *Salmonella* from different animal sources. As an example of a strikingly rapid emergence, data on the occurrence of resistance to cefotaxime, defined by the epidemiological cut-off value of EUCAST (>0.25 mg/l), in *E. coli* from healthy broilers, and in *Salmonella* Paratyphi B var. Java from broilers in the Netherlands are shown in Figure 3 (data from MARAN 2005; Dik Mevius, personal communication, 2007).

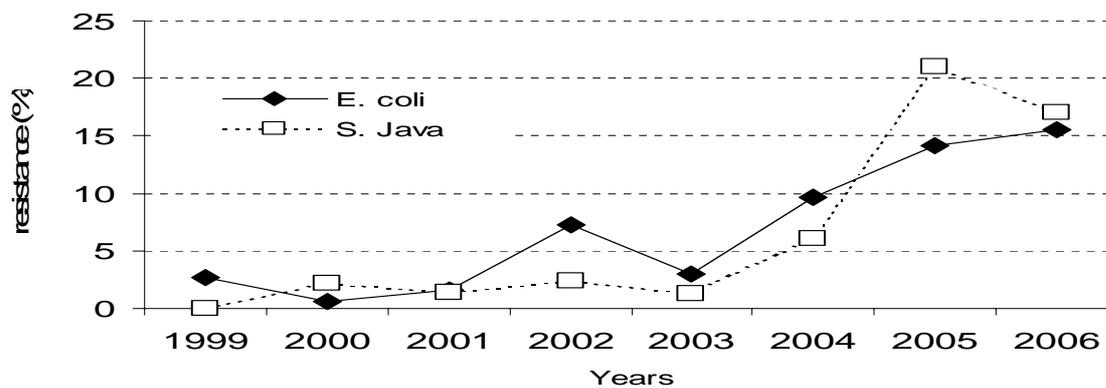


Figure 3. Occurrence of resistance to cefotaxime in *E. coli* and *Salmonella* Paratyphi B var. Java from broilers in the Netherlands.

The resistance data in Tables 3 and 4 consist of percentages of resistance among bacterial isolates investigated, i.e. the epidemiological unit of concern is the bacterial species colonising a particular animal. By use of selective screening techniques, data on prevalence on animal-level can be obtained. In studies where such techniques have been used, the prevalence of pigs and poultry carrying *E. coli* with decreased susceptibility to 3<sup>rd</sup> generation cephalosporins varies widely, from 10% to 93% (Girlich *et al.*, 2007; Jorgensen *et al.*, 2007; Moreno *et al.*, 2007). Even in cases when the prevalence of animals with *E. coli* showing decreased susceptibility was high, only a low percentage of *E. coli* in each sample displayed this trait.

Since laboratory detection of these types of resistance can be problematic and underreporting may have occurred, the information above indicates that the resistance to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins in *E. coli* and *Salmonella* from animals is rapidly emerging in some European countries. In others information is still incomplete and trends are difficult to assess.

### Emergence of ESBLs

Non-typhoidal *Salmonella* spp with ESBL-type resistance appeared in the late 80s early 90s. Subsequently, the number serovars, enzymes and countries involved have increased steadily (Arlet *et al.*, 2006; Miriagou *et al.*, 2004). In 2004, various ESBLs of the SHV, TEM or CTX-M families had been described in more than 30 serovars isolated from humans or animals in more than 30 countries, whereof 13 European (Arlet *et al.*, 2006). Judging by the number of reports, in Europe TEM-52, SHV-2, -5 and -12 and a wide array CTX-M-enzymes are the most commonly encountered (Arlet *et al.*, 2006). This is also true if only reports including isolates from food producing animals or food are included.

In a comprehensive study from the Netherlands including non-duplicate isolates of *Salmonella* from poultry and poultry meat from 2001-2002, Hasman *et al* (2005) showed a great diversity of serovars and ESBLs. TEM-52 was the most common ESBL, occurring in various serovars including *S. Enteritidis* and *S. Typhimurium*. In 2002, a multiresistant *Salmonella* Virchow producing CTX-M-9 was isolated from a girl in France and similar isolates were recovered in 2003 from six chicken farms and one hatchery supplying these farms, as well as from poultry meat at retail (Weill *et al.*, 2004). Production of CTX-M-9 was also reported from Spain in *S. Virchow* and *S. Enteritidis* isolated in 2003 and 2004, a study that also reported SHV-12 in a *S. Rissen* isolated from a pig (Riano *et al.*, 2006). From Greece, multiresistant *S. Virchow* producing CTX-M-32 were isolated in 2001 from two batches of poultry products from the same company (Politi *et al.*, 2005). The clonal emergence during 2000-2003 of a multiresistant *S. Virchow*-producing CTX-M-2 was described from Belgium and France (Bertrand *et al.*, 2006). An isolate of *S. Virchow* with a similar antibiogram was also described from poultry (isolated 2002) in the study from the Netherlands (Hasman *et al.*, 2005).

A series of publications based on materials from the Spanish Veterinary-Antimicrobial-Resistance-Surveillance (VAV) Network describe an increase in the percentage and variety of ESBLs (and of AmpC-type resistance, see below) over time. In a study on *E. coli* isolated from healthy animals and foods in Spain during 1997-1999, genes encoding TEM, SHV and OXA were demonstrated. Some of the enzymes were inhibitor resistant, but none were of ESBL-type (Brinas *et al.*, 2002). In a study on isolates from 2000-2001 from healthy chickens, one isolate carrying a gene encoding CTX-M-14 was detected (Brinas *et al.*, 2003b). During year 2003, several CTX-M-variants (CTX-M-1, CTX-M-9, CTX-M-14 and CTX-M-32) and SHV-12 were demonstrated in *E. coli* from materials from sick food producing animals and from healthy chickens (Brinas *et al.*, 2005). The strains carrying genes encoding CTX-M-type enzymes appeared to be clonally unrelated. In a separate study on *E. coli* isolated from Catalonian poultry, pig and rabbit farms various CTX-M-type enzymes were demonstrated as well as SHV-2 and TEM-52 (from poultry) (Blanc *et al.*, 2006). Different patterns were observed depending on animal species, and as in the previous study, the strains showed a low clonal relationship. The lack of clonality indicates horizontal spread of plasmids or other transferable genetic elements. In human medicine, dissemination of genes encoding CTX-M-9 is associated with the large conjugative plasmids carrying often also conferring resistance to aminoglycosides and trimethoprim. Similarly, epidemic plasmids carrying genes encoding CTX-M-14 or CTX-M-32 have been described (Canton & Coque, 2006; Livermore *et al.*, 2007).

From the UK, the first isolates of *E. coli* producing a CTX-M-14-like enzyme were reported from diarrhoeic calves in 2005 (Teale *et al.*, 2005). In France, CTX-M-1 and CTX-M-15 were detected in clinical isolates from cattle, swine and poultry (Meunier *et al.*, 2006) and in Portugal from broilers (Pomba *et al*, 2008). Further, in a screening study using selective techniques, 12 of 112 healthy poultry sampled at slaughter carried CTX-M-1 producing *E. coli* (Girlich *et al.*, 2007). In Belgium, CTX-M-1 was also the most common ESBL enzyme among healthy broilers at five commercial farms, but also TEM-52, CTX-M-2, CTX-M-14 and TEM-106 were also present (Smet *et al*, 2008). In a Portuguese study, TEM-52, SHV-2 and CTX-M-1 were detected in *Enterobacteriaceae* in samples from chicken meat and broilers and SHV-12 in samples from healthy swine (Machado *et al*, 2008). CTX-M-1 has also been described in clinical isolates of *E. coli* and *Klebsiella pneumoniae* from horses in the Netherlands (Vo *et al.*, 2007). Occurrence of CTX-M-2 and CTX-M-18 in *E. coli* from healthy chickens and CTX-M-2 from cattle has been reported from Japan (Kojima *et al.*, 2005; Shiraki *et al.*, 2004).

Taken together, a wide array of genes encoding ESBLs has emerged and is now present in enteric bacteria from animals, although the different enzymes found in animals are markedly less diverse than those found in isolates from humans. In particular, different CTX-M type genes are increasingly reported both in *E. coli* and in *Salmonella* from food producing animals in Europe in recent years. As noted previously, the genes encoding ESBLs are often encoded on plasmids and/or other transferable genetic elements and are often linked to multiple other resistance genes. Spread of resistance can be clonal or horizontal, or both.

## Emergence of transferable AmpC-type beta-lactamases

The first occurrence of plasmid mediated AmpC-type beta-lactamase (CMY-2) was described from humans in Algeria in an isolate of *S. Senftenberg* from 1994 (Koeck *et al.*, 1997). Later, AmpC production was reported in a multidrug resistant *Salmonella*-isolate in the US (Horton *et al.*, 1999). This was rapidly followed by a number of reports from the US and Canada on production of CMY-2 by different serovars of *Salmonella* spp isolated from animals and food, in particular in *S. Typhimurium* and *S. Newport* (Alcaine *et al.*, 2005; Allen & Poppe, 2002; Carattoli *et al.*, 2002; Chen *et al.*, 2004; Fey *et al.*, 2000; Gray *et al.*, 2004; Pitout *et al.*, 2003; White *et al.*, 2001; Winokur *et al.*, 2000; Winokur *et al.*, 2001; Zhao *et al.*, 2003). In most reports, the isolates were multiresistant (Biedenbach *et al.*, 2006). From 1999 to 2003, resistance to ceftiofur in *Salmonella* increased from 4% to 19%, with *S. Newport* being the most common serovar (Frye & Fedorka-Cray, 2007). Resistance was predominantly associated with CMY-2 encoding plasmids. Available information indicates that the increase of MDR-AmpC *S. Newport* is explained by the spread of one clone among animals and humans (Berge *et al.*, 2004; Zhao *et al.*, 2003).

There are to date only two reports from Europe on production of CMY-2 in *Salmonella* from animals, and in both cases there was a link to imported animals (Aarestrup *et al.*, 2004; Liebana *et al.*, 2004). Likewise, in an outbreak of MDR-AmpC *S. Newport* in France, imported horsemeat was implicated (Espie *et al.*, 2004). Another AmpC-type enzyme, ACC-1, was described in *Salmonella* spp in a study from the Netherlands (Hasman *et al.*, 2005). The gene encoding ACC-1 was present in *S. Bareilly*, *S. Braenderup* and *S. Infantis* and it was carried on indistinguishable plasmids.

In the US, plasmid-mediated genes encoding CMY-2 appear to be widely disseminated in *E. coli* from diseased and healthy food producing animals and food (Bradford *et al.*, 1999; Donaldson *et al.*, 2006; Winokur *et al.*, 2001; Zhao *et al.*, 2001). In one of these studies, 15% of 377 isolates of *E. coli* from clinical submissions from cattle and pigs were carrying CMY-2 (Winokur *et al.*, 2001). More than 90% of these isolates were also resistant to tetracycline, sulfonamides and streptomycin, almost 70% to gentamicin and 15% to ciprofloxacin (resistance defined by clinical break-points).

In a Belgian study on occurrence of ceftiofur-resistance among *E. coli* isolated from broilers at five farms, CMY-2 was as common as ESBL-type enzymes. Eight percent of the ceftiofur-resistant isolates had both CMY-2 and CTX-M-1 (Smet *et al.*, 2008). Plasmid-mediated AmpC-type beta-lactamases have been reported from Spain and Portugal at low frequency in *E. coli* isolated from diagnostic submissions from cattle, pigs and broiler (Brinas *et al.*, 2003a, Pomba *et al.* 2008) and from Spain from healthy chickens and rabbits (Blanc *et al.*, 2006; Brinas *et al.*, 2005). Batchelor *et al.* (2005a) reported the isolation of CMY-2 positive *E. coli* from one of 140 samples from healthy cattle in the UK. Two different types of *E. coli* harbouring indistinguishable large CMY-2 carrying plasmids were isolated from the same animal. In The Netherlands, *E. coli* with plasmid mediated CMY-2 has recently been reported in a clinical isolate of *E. coli* from a horse (Vo *et al.*, 2007). Plasmid mediated CMY-2 have also been reported from healthy food producing animals in Japan (Kojima *et al.*, 2005).

In summary, plasmid mediated CMY-2 resistance has become widespread among *Salmonella* and *E. coli* in animals in North America. In Europe, most reports indicate a limited occurrence, but high frequencies in a recent study on broilers (Smet *et al.*, 2008) indicate that also this type of enzymes may be increasing. As for the ESBLs, the plasmid borne genes encoding CMY-2 are mostly linked to multiple other resistance genes. Spread of resistance can be clonal or horizontal, or both.

## INFLUENCE OF USE OF ANTIMICROBIALS ON THE EMERGENCE AND SPREAD OF RESISTANCE

Following systemic administration, ceftiofur and cefquinome are mainly excreted in urine with a limited portion, excreted in faeces (EMA/MRL/005/95, Summary of Product Characteristics, Annex 1; Naxcel 100 mg/ml). Information on what that means in terms of active concentrations over time in intestinal contents is not available in sources in the public domain. Such information is essential to evaluate the exposure of the gastro-intestinal microbiota of the target animal to the parent drug or active metabolites (CVMP/VICH GL 27; Guidance on the pre-approval information for registration of new veterinary medicinal products for food producing animals with respect to antimicrobial resistance, CVMP/VICH/644/01-final).

### **Influence of cephalosporin use on occurrence of MRSA**

As MRSA are resistant to all beta-lactams, use of any substance in that group may provide selective pressure. In human medicine, use of cephalosporins, other beta-lactams, fluoroquinolones and glycopeptides have been shown by metanalysis to be associated with an increased risk of acquisition of MRSA (Taconelli *et al*, 2008). In view of the increasing occurrence of MRSA in animals, the risk associated with use of substances with a potential to select for MRSA-colonisation of animals should be further examined. The potential influence of the use of products formulated as 'long acting', with long excretion times deserve special attention, as the time when concentrations are close to the MIC of intestinal and skin microbiota can be long. This document is focused on resistance with particular relevance for the 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins rather than all cephalosporins and other penicillinase stable beta-lactams. Emphasis will, therefore be on resistance in Gram-negative enteric bacteria and MRSA will not be further discussed in detail.

### **Influence of cephalosporin use on the evolution of genes encoding beta-lactamases**

The beta-lactamases TEM-1 and SHV-1 are common in bacteria from various animals. These enzymes do not confer resistance but mutations in the genes encoding these enzymes lead to structural changes that can extend or alter the substrate specificity (Gniadkowski, 2008). Similarly, mutations in the genes encoding AmpC-type enzymes can give rise to extended spectrum AmpC with activity also against 4<sup>th</sup> generation cephalosporins (Ahmed & Shimamoto, 2008; Le Turnier *et al*, 2008, Mammeri *et al.*, 2007; Mammeri *et al*, 2008; Wachino *et al*, 2006).

The evolution of ESBLs has been attributed to the selective pressure exerted by use of higher generation cephalosporins (Medeiros, 1997). There are a number of studies in human clinical settings in support of that (Gniadkowski, 2008). Blázquez *et al* (2000) have suggested a broader view: that *in vivo* evolution of ESBLs is driven by the constant fluctuating pressure of various beta-lactams, including also penicillins and first generation cephalosporins. This may explain why many of the enzymes generated *in vitro* never occur naturally – only ESBLs with a truly broad-based resistance would survive and be selected for in an environment where different beta-lactams are used.

Current knowledge on use of cephalosporins as a driver of the evolution of ESBLs and AmpCs is based on laboratory studies and studies in human clinical settings. It is probable that the general principle applies also to animal production thus the use of 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins in animal populations, and possibly also of other beta-lactams, may favour the evolution of beta-lactamases in exposed bacterial populations.

### **Influence of cephalosporin use on selection and amplification of genes encoding beta-lactamases**

Use of 3<sup>rd</sup> generation cephalosporins is a recognised risk factor for ESBL colonisation of patients in the human hospitals (Asensio *et al.*, 2000; Quale *et al.*, 2002; Saurina *et al.*, 2000; Urbanek *et al.*, 2007). Several authors have suggested that the use ceftiofur in cattle and turkeys may have contributed to the spread in *Salmonella* in North America, of plasmid mediated AmpC-type beta-lactamases (Allen

& Poppe, 2002; Dunne *et al.*, 2000; Fey *et al.*, 2000; White *et al.*, 2001; Winokur *et al.*, 2000). Until recently there have been no specific studies on the influence of the use of 3<sup>rd</sup> generation cephalosporins on resistance in *Enterobacteriae* in food producing animals.

In an experimental study administration of a single dose of ceftiofur to turkey poult without detectable ceftiofur-resistant strains did not result in the emergence of resistant strains (Poppe *et al.*, 2005). In a parallel experiment, the poult were dosed both with susceptible *S. Newport* and with *E. coli* carrying a large plasmid encoding AmpC-type beta-lactamases. The plasmid was readily transferred in the intestine to the *Salmonella* strain and also to a serotype of *E. coli* different from the donor in absence of any selective pressure. The experiment did not include a group receiving both antimicrobials and bacteria carrying resistance; hence the influence of ceftiofur on transfer and shedding of bacteria carrying resistance genes was not evaluated.

In a small experimental study, pigs were injected with ceftiofur, cefquinome or amoxicillin once daily for three days (Cavaco *et al.*, 2008). Untreated animals served as controls. Almost all animals were positive for *E. coli* with CTX-M-1 before the start of the experiment. Animals were also inoculated with a CTX-M-1 producing strain of *E. coli* before the start of treatments. Significantly higher counts of cefotaxime-resistant coliforms were observed in all treated groups compared with controls for up to 22 days after the end of treatment. The cephalosporins had a more pronounced effect than amoxicillin.

Tragesser *et al.* (2006) studied the occurrence of ceftriaxone-resistant *E. coli* in dairy herds in Ohio, USA and linked the results to information on use of ceftiofur in the studied herds. Most of the isolates that showed reduced susceptibility to ceftriaxone carried a plasmid coding for CMY-2. Such isolates were recovered from at least one of the sampled cows in 10 of the 12 herds reporting use of ceftiofur, and in 2 of 7 herds reporting non-use (Odds ratio 25, P=0.01 add confidence intervals). The mean within-herd prevalence was 40% for herds reporting use of ceftiofur, compared to 9% for those reporting non-use. There was no association at individual cow-level, nor was there a linear relation between within-herd prevalence and treatment frequency. There was no attempt to analyse the influence of use of other antimicrobials on the farm. All CMY-2 producing isolates of *E. coli* were co-resistant to streptomycin, sulphonamides and tetracycline, and in addition, commonly also to gentamicin, kanamycin and trimethoprim-sulphonamides. Co-selection by other antimicrobials as well as management factors could account for the lack of linear relation between within-herd prevalence and use of ceftiofur.

In a study from Denmark, pigs in farms using and not using ceftiofur were sampled (Jorgensen *et al.*, 2007). *E. coli* with reduced susceptibility to 3<sup>rd</sup> generation cephalosporins was demonstrated in 69 of 200 sampled pigs (5 of 10 farms) but only in 3 of 200 animals in control farms (1 of 10 farms). The difference was statistically significant (P=0.02). Production of ESBL (CTX-M-1) was demonstrated in 19 isolates from two of the ceftiofur-using farms (not statistically different from farms not using ceftiofur). The study did not examine other drug use practices in the farms.

Lowrance *et al.* (2007) studied the influence of administration of ceftiofur crystalline free acid ('long acting') to steers. Ceftiofur was administered subcutaneously to different cohorts at 6.6 mg/kg, 4.4 mg/kg (single doses) and at 6.6 mg/kg three times with 6 days interval). Untreated steers served as controls. Ceftiofur-resistant faecal *E. coli* were present at the start of the study, and administration of ceftiofur was associated with an increase in the proportion of *E. coli* resistant to ceftiofur during treatment in all treated groups. No changes in proportion of resistant isolates recovered using non-selective techniques were observed in co-mingled control animals. Almost all resistant isolates were co-resistant to at least chloramphenicol, streptomycin, sulphonamides and tetracyclines, a pattern associated with a multiresistance plasmid described in AmpC-producing *Salmonella* and *E. coli* (Winokur *et al.*, 2001).

The influence of general use of antimicrobials on antimicrobial resistance in bacteria from calves in the US was studied in a field trial (Berge *et al.*, 2006). Individual treatments transiently increased the

shedding of multiply-resistant *E. coli* compared with non-treated calves. The isolates were resistant to ceftiofur, which was the antimicrobial used for most of the individual treatments.

A longitudinal study over five months on healthy young calves on a dairy farm showed a persistent high prevalence (65-100%) of calves shedding ceftiofur-resistant, CMY-2 producing, *E. coli* (Donaldson *et al.*, 2006). The isolates were all multi-resistant and belonged to 59 clonal types. The farm reported use of various antimicrobials including ceftiofur but kept no individual records; thus no attempt to correlate use with resistance could be made.

The persistence of ESBLs of CTX-M type on a dairy farm in the absence of use of cephalosporins and other beta-lactams has been documented from the UK (Liebana *et al.*, 2006). Although, all use of beta-lactam antimicrobials, apart from intramammary use of cefquinome, was stopped during the study period in an attempt to remove the selective pressure. The prevalence of animals shedding CTX-M positive *E. coli* remained high over 6 months. As in studies on CMY-2, there was a diversity of clones but an almost complete predominance of one plasmid type carrying a gene encoding CTX-M and in addition, streptomycin resistance. Occurrence of *E. coli* producing ESBL- and CMY- in the apparent absence of use of cephalosporins has also been reported in broiler (Smet *et al.*, 2008, MARAN 2005).

It has been argued that active concentrations of ceftiofur in the intestines of treated animals are very low, and that the substance is rapidly metabolised by the intestinal microbiota (Hornish & Kotarski, 2002). The studies quoted above show that the concentrations are sufficient to select for *E. coli* with resistance to 3<sup>rd</sup> generation cephalosporins. The lack of clonality of resistant isolates reported in several studies clearly indicates horizontal dissemination of resistance genes.

### **Co-selection of resistance in *Enterobacteriaceae* by non-cephalosporin antimicrobials**

As discussed above, ESBL- or AmpC- producing bacteria are often also resistant to multiple other antimicrobials. In most cases, the genes encoding these unrelated resistance traits are linked on the same plasmid or transferable genetic element as the ESBLs. Many of the antimicrobials in question are used in veterinary medicine, e.g. neomycin, streptomycin, tetracycline, trimethoprim, sulphonamides and fluoroquinolones. A few of these substances are also used as growth promoters in some parts of the world. Of particular concern is the described association between CTX-M or AmpC encoding genes and plasmid-mediated quinolone resistance (Robicsek *et al.*, 2006a). In human hospitals, use of fluoroquinolones has been identified as a risk factor for spread of CTX-M (Ben-Ami *et al.*, 2006).

The frequent linkage of resistance genes implies that once the ESBL- or CMY- encoding genes have entered a bacterial population in a production unit, a broad range of antimicrobials, including beta-lactams such as amoxicillin, but also of structurally unrelated antimicrobials can favour their selection and spread between animals and between bacterial strains (co-selection). In The Netherlands, *Salmonella* and *E. coli* producing ESBL have emerged and increased in prevalence in poultry without prior use of cephalosporins (MARAN, 2005). Similarly, a high prevalence of ESBL- and CMY- producing *E. coli* has been reported in Belgian broiler flocks (Smet *et al.*, 2008). It is probable that use of either beta-lactams such as amoxicillin, or non-beta-lactam antimicrobials, have contributed to the observed increase.

## **EXPOSURE OF HUMANS TO RESISTANT BACTERIA AND RESISTANCE GENES FROM ANIMAL SOURCES**

### **Exposure to resistant bacteria from animals**

As noted previously occurrence of ESBL- or AmpC- producing *Salmonella* of different serovars from animals and from food of animal origin has been demonstrated in a number of studies (for references see previous sections).

Most salmonella infections in humans are attributed to food-borne transmission. Person-to-person transmission is uncommon, except in outbreaks with nosocomial spread. Therefore exposure of humans to *Salmonella* resistant to cephalosporins via food, direct contact with infected animals or indirectly via the environment will have a significant influence on the occurrence of ESBL- or AmpC-producing *Salmonella* in humans. This is supported by the observation in the US of a temporal association between emergence of AmpC-type beta-lactamases in *Salmonella* from various animals and an increased prevalence of such infections in humans (Frye & Fedorka-Cray, 2007; Gupta *et al.*, 2003; Lopes *et al.*, 2006). Several outbreaks of cephalosporin-resistant *Salmonella* (AmpC or ESBL producing) have been linked to consumption of animal products (Bertrand *et al.*, 2006; Espie *et al.*, 2004; Weill *et al.*, 2004). An outbreak of human infections with multiresistant, CMY-2-producing *S. Newport* implicating handling of pet treats containing dried beef as the source has been described in Canada (Pitout *et al.*, 2003).

Direct spread of a multiresistant *S. Newport*, also resistant to 3<sup>rd</sup> generation cephalosporins, from cattle to a person was documented by Fey *et al.* (2000). In a retrospective case-control study, patients infected with multidrug-resistant (including AmpC-type resistance) *S. Newport* were more likely to have had direct contact with cattle than either patients infected with susceptible *S. Newport* or matched healthy controls (Gupta *et al.*, 2003).

Taken together, it is clear humans are exposed to cephalosporin-resistant *salmonella* via food or via direct contact with infected animals, and that this may result in clinical infections. This is also discussed in a recently published opinion by EFSA (Anonymous, 2008)

### **Exposure to resistance genes from bacteria associated with food of animal origin**

In the study by Corpet (1988), volunteers eating sterilised food had significantly less coliforms resistant to tetracyclines in their faeces than when eating non-sterilized food. This type of study has not been repeated since, but data on occurrence of resistant *E. coli* on food, including vegetables, indicate that humans ingest resistant bacteria on a daily basis. During the passage through the intestine, such bacteria may transfer their resistance genes to host- adapted bacteria or to zoonotic pathogens. Exchange of resistance genes between bacteria from different sources has also been demonstrated in water, soil, on kitchen towels, on cutting boards and on the surface of food (Kruse & Sørum, 1994). Evidence for horizontal transfer of plasmids or resistance genes other than cephalosporin resistance between bacteria colonising animals and those colonising humans has been documented in several studies (Chaslus-Dancla *et al.*, 1991; Hunter *et al.*, 1994; Lester *et al.*, 2006; Levy *et al.*, 1976; Nikolich *et al.*, 1994; Tschäpe, 1994).

Genes encoding ESBL- or AmpC- type resistance have been demonstrated, not only in *Salmonella* isolated from food (see above) but also in *E. coli* (Brinas *et al.*, 2002; Zhao *et al.*, 2001). As discussed, these genes are mostly carried on mobile genetic elements. The number of studies is still limited, as is information on prevalence of resistance to cephalosporins in *E. coli* isolated in meat in Europe (see Table 3). However available information suffices to conclude that humans can be exposed to genes encoding ESBL or AmpC-type resistance via food.

Indistinguishable plasmids or other genetic elements coding for ESBLs or AmpC-type resistance have been described from different bacterial species and different animal and human hosts (Batchelor *et al.*, 2005a; Hasman *et al.*, 2005; Poppe *et al.*, 2005; Winokur *et al.*, 2001). Thus, there is evidence that the plasmids carrying genes encoding ESBLs and AmpC-type beta-lactamases are transferred horizontally between different bacterial species of different hosts. Certain plasmids carrying genes encoding CMY-2 are disseminated among both *Salmonella* and *E. coli* from both animals and humans, and the pattern indicates that certain plasmids are epidemic (Hopkins *et al.*, 2006, Mulvey *et al.*, 2008). Further, there are some reports indicating acquisition of resistance plasmids by *E. coli* and *Salmonella* in the human gut (Su *et al.*, 2003; Yan *et al.*, 2005). A plasmid encoding ESBL was identified in *E. coli* and *S. Anatum*, both from the same patient. As the resistant isolates had molecular fingerprints identical to those of susceptible isolates of the same species isolated earlier from the same patient, it was

concluded that the acquisition of the same plasmid by two different bacteria had probably occurred in the gut (Su *et al.*, 2003). With similar type of evidence, Yan and co-workers reported on a *S. Hadar* with AmpC-type resistance apparently acquired from an *E. coli* from the same patient (Yan *et al.*, 2005).

In summary, bacteria of animal origin carrying resistance genes encoding ESBL or AmpC can be present in food. Moreover, at that stage cross-contamination between food-items and human contamination through food processing might also play a role (Anonymous, 2008). Transfer of such genes to bacteria causing disease in humans can occur in the intestine. The present extent of exposure via food is difficult to determine. Any further expansion of the occurrence of ESBL or AmpC resistance among animal bacteria is likely to have an influence on the occurrence in food, and thereby on human exposure.

## IMPACT OF INFECTIONS WITH CEPHALOSPORIN-RESISTANT BACTERIA ON HUMAN AND ANIMAL HEALTH

### Human health

Gastroenteritis is the most common clinical manifestation of *Salmonella* infections, but severe cases with systemic manifestations occur. In those cases antimicrobial treatment is often recommended. Serious infections are most common in children and elderly (Arlet *et al.*, 2006). Among the first line empiric treatments for adults are the fluoroquinolones. In very young patients and when fluoroquinolone resistance is present, 3<sup>rd</sup> generation cephalosporins are the drugs of choice.

In a study of *S. Newport* infections, no significant differences in symptoms, hospitalisation, duration of illness or other outcomes between patients infected with susceptible isolates and isolates of MDR-AmpC resistance phenotype could be demonstrated (Devasia *et al.*, 2005). The lack of demonstrable impact of the multiresistance phenotype was probably a consequence of empiric treatment mostly done with fluoroquinolones, to which the isolates were susceptible. Infections of humans with *Salmonella* resistant to both cephalosporins and ciprofloxacin have been described (Cheung *et al.*, 2005; Chiu *et al.*, 2004; Ko *et al.*, 2005; Yan *et al.*, 2005), in some cases in association with fatalities. The emergence of multiresistant *Salmonella* with additional resistance to cephalosporins and fluoroquinolones seriously limits the therapeutic options available.

The recent and rapid emergence of resistance of CTX-M type ESBLs in *Enterobacteriaceae* from human infection in Europe is of major public health concern (Canton & Coque, 2006; Livermore *et al.*, 2007). The increasing frequency of community-acquired infections and in particular of infections with *E. coli* resistant to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins is of concern. Prevalence data was reviewed by Rodriguez-Bano and Navarro (2008). For example, in Seville, the percentage of *E. coli* that was ESBL producers increased from 0.3% in 1995-1998 to 4.8% in 2002. Approximately half of these isolates were from non-hospitalised patients. The percentage of ESBL producing *E. coli* among isolates from community-acquired urinary tract infections ranged from 1.5% to 3.3% in studies from Seville, Barcelona and the Gaza strip. In a multicentre study from Spain, 3.7% of *E. coli* isolated in 2003 from community-acquired intra-abdominal infections were ESBL-producers. Two Spanish studies illustrate that the recorded increase in infections was matched by an increase in faecal carriage in outpatients and healthy volunteers (Mirelis *et al.*, 2003; Valverde *et al.*, 2004).

Many patients with community-acquired infections have a history of hospitalisation and have co-morbidities, but cases of uncomplicated cystitis also occur. The routes of spread of genes encoding CTX-M outside hospitals are still not clear, but the epidemiological pattern indicates that reservoirs may exist in the community (Livermore *et al.*, 2007). Considering the emergence of CTX-M producing *Salmonella* and *E. coli* in animals as discussed above, it has been hypothesized that animals may be one of several potential reservoirs and food a potential vector (Livermore *et al.*, 2007).

The therapeutic options for infections with bacteria resistant to 3<sup>rd</sup> generation cephalosporins are limited. In particular, this is true for community-acquired infections where oral therapy is preferred (Canton & Coque, 2006). Theoretically one option is fluoroquinolones, but as CTX-M type resistance is frequently linked to other resistance determinants such as plasmid encoded quinolone-resistance, there is a high likelihood that such antimicrobials is not an effective alternative.

### **Animal health**

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 3<sup>rd</sup> or 4<sup>th</sup> generation cephalosporins could be the sole alternative 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 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins (Rantala et al., 2002; Erskine *et al.*, 2002; Shpigel *et al.*, 1997, Poutrel *et al* 2008). 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 *et al*, 2008). In many countries, resistance to both gentamicin and trimethoprim-sulphonamides in the Gram-negative target pathogens exist. In such cases, 3<sup>rd</sup> or 4<sup>th</sup> 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 antimicrobial agents. A further increase of cephalosporin resistance can indirectly impact on animal health by increasing the prevalence of multiresistance, thereby severely reducing the number of effective alternatives for treatment.

## SUMMARY ASSESSMENT

### **Resistance to 3<sup>rd</sup> generation cephalosporins in e.g. *K. pneumoniae* and *E. coli* in human infections is increasing in Europe.**

Genes coding for CTX-M type enzymes have rapidly emerged and spread not only in hospitals but also in the community. Production of ESBLs in *Enterobacteriaceae* is often associated with resistance to other antimicrobials. The changing epidemiological pattern may be explained by many interacting factors. Many problems in human medicine can be correlated to use of cephalosporins and other antimicrobials in humans, but it is possible that spread from animal reservoirs via food or via the environment may contribute to the dissemination of resistance in the community. The potential role of community reservoirs of animal origin such as food of different origins, and of other potential reservoirs such as the environment needs further investigation.

### **Available data indicate that resistance to 3<sup>rd</sup> generation cephalosporins is increasing in *E. coli* and *Salmonella* from animals in Europe.**

Many countries still report low or zero prevalence but others have noted pronounced increases. A wide array of genes encoding ESBLs has emerged and is now present in enteric bacteria from animals. The occurrence of resistance to cephalosporins among bacteria isolated from animals may have been underestimated in the past, both because of methodological weaknesses and use of insensitive interpretation criteria.

Work aiming to harmonise methodology and interpretation criteria is undertaken by EFSA. Better data will be available if monitoring of resistance to 3<sup>rd</sup> generation cephalosporins in the zoonosis-monitoring programmes is conducted, in accordance with the standards developed by EFSA. Furthermore an expansion of the monitoring to include commensal *E. coli* from animals and food from all Member States would provide data valuable for the assessment the reservoir of resistance genes.

### **The genes encoding resistance to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins are transferrable and often linked to other resistance genes.**

A wide array of genes encoding ESBLs has emerged and is now present in enteric bacteria from animals. The genes encoding ESBLs are often encoded by plasmids and/or other transferable genetic elements, and are often linked to other resistance genes. Spread of resistance can occur both through dissemination of clones, and through horizontal transmission of, e.g. epidemic plasmids.

### **Data on the extent of use of 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins for animals in the EU is not presented in a way that allows exposure to be properly assessed.**

More information is needed on the influence of the use of cephalosporins in veterinary medicine on the evolution of new variants of ESBLs, and on potential differences between different doses and dosing regimens in this respect. It is important that the use of antimicrobials will be monitored in a way that allows for the use of different generations of cephalosporins per animal species to be followed.

### **Systemic use of 3<sup>rd</sup> and 4<sup>th</sup> cephalosporins selects for resistance.**

The use of 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins can influence resistance in two ways: either by favouring the evolution of new variants of ESBL genes by selecting for emerging mutants, or by selecting for genes that have been introduced from other sources into the exposed population. Excretion of the drug into the intestine after systemic administration is low, but data on exact concentrations are not easily available. A relation between use of ceftiofur and occurrence of resistance at herd level has been documented, showing that the concentrations are high enough to select for resistance.

**Co-selection by other antimicrobials is likely to influence prevalence of resistance to 3<sup>rd</sup> and 4<sup>th</sup> cephalosporins.**

The emergence of cephalosporin resistance has been documented in poultry production systems where no cephalosporins are authorised for use. Resistance may also persist in farms in the absence of systemic use of beta-lactams. The genes encoding ESBL or AmpC-type enzymes are frequently linked to genes conferring resistance to other, unrelated antimicrobials. It is, therefore likely that co-selection by antimicrobials other than cephalosporins have an important role. In particular, medication of large groups of animals (mass medication) with various antimicrobials in animal husbandry may contribute to the occurrence and dissemination of resistance in exposed populations.

The importance of use of non-cephalosporin antimicrobials for selection and maintenance of cephalosporin resistant bacteria in animal populations needs to be documented further.

**Humans are exposed to cephalosporin-resistant bacteria via food or via direct contact with infected animals or indirectly through the environment.**

Humans are exposed to cephalosporin-resistant *Salmonella* via food or via direct contact with infected animals, and this can result in clinical infections. Furthermore, humans may be exposed to animal bacteria with resistance genes coding for ESBLs or AmpC type enzymes via direct contact, via contaminated food or indirectly through the environment. These genes can be transferred to bacteria with potential to cause infections in humans. The extent of such exposure is difficult to determine. It is related to the prevalence of such genes in bacteria colonising animals, but will also be influenced by other factors, e.g. those related to food hygiene and consumption habits.

**In human medicine, the options for effective treatments of infections that are resistant to 3<sup>rd</sup> and/or 4<sup>th</sup> generation cephalosporins are limited.**

In the case of infections in humans with *Salmonella*, *E. coli* or other *Enterobacteriaceae* that are resistant to 3<sup>rd</sup> and/or 4<sup>th</sup> generation cephalosporins, treatment alternatives are e.g. carbapenems, fluoroquinolones or aminoglycosides. The occurrence of co-resistance often seriously limits the options for effective treatment and some of the alternatives carry a high risk of adverse effects or are difficult to use in outpatient settings.

To conclude, resistance to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins is rapidly increasing in humans. Available evidence indicates that resistance to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins is also emerging in animal populations. Although there are many uncertainties, the potential consequences of a further increase of ESBL and AmpC type resistance in bacteria colonising animals are serious. Measures to counter a further increase and spread of resistance in animals should therefore be considered.

## REFERENCES

- Aarestrup, F. M., Hasman, H., Olsen, I., and Sorensen, G. International spread of bla<sub>CMY-2</sub> mediated cephalosporin resistance in a multiresistant *Salmonella enterica* serovar Heidelberg isolate stemming from the importation of a boar by Denmark from Canada. *Antimicrobial Agents and Chemotherapy*. 2004, **48**, 1916-17
- AFSSA. Suivi des ventes de médicaments vétérinaires contenant des antibiotiques en France en 2005. <http://www.afssa.fr>
- Ahmed AM, Shimamoto T. Emergence of a cefepime- and ceftiofime-resistant *Citrobacter freundii* clinical isolate harbouring a novel chromosomally encoded AmpC beta-lactamase, CMY-37. *International Journal of Antimicrobial Agents*. 2008, **32**, 256-61.
- Alcaine, S. D., Sukhnanand, S. S., Warnick, L. D., Su, W.-L., McGann, P., McDonough, P., and Wiedmann, M. Ceftiofur-resistant *Salmonella* strains isolated from dairy farms represent multiple widely distributed subtypes that evolved by independent horizontal gene transfer. *Antimicrobial Agents and Chemotherapy*. 2005, **49**, 4061-7
- Allen, K. J., and Poppe, C. Occurrence and characterization of resistance to extended spectrum cephalosporins mediated by beta-lactamase CMY-2 in *Salmonella* isolated from food-producing animals in Canada. *Canadian Journal of Veterinary Research*. 2002, **66**, 137-44
- Alvarez, M., Tran, J. H., Chow, N., and Jacoby, G. A. Epidemiology of conjugative plasmid-mediated AmpC beta-lactamases in the United States. *Antimicrobial Agents and Chemotherapy*. 2004, **48**, 533-7
- Ambler, R. P. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci*. 1980, **289**, 321-31
- Anonymous. EARSS (European Antimicrobial Resistance Surveillance System) Annual report 2005. 2006a. <http://www.rivm.nl/earss>.
- Anonymous. Foodborne antimicrobial resistance as a biological hazard. Scientific opinion of the panel of biological hazards. *The EFSA Journal* 2008, **765**, 2-87. <http://www.efsa.europa.eu>
- Anonymous. Guidelines for ATC classification and DDD assignment. WHO Collaborating Centre for Drug Statistics Methodology. 2005. <http://www.whocc.no/atcddd/>
- Anonymous. Memorandum 2003:9a: Recommendations for the use of antimicrobial agents in the treatment of the most significant infectious diseases in animals, Memorandum to the Ministry of Agriculture and Forestry, Helsinki. 2003. [http://wwwb.mmm.fi/julkaisut/tyoryhmamuistiot/2003/tr2003\\_9a.pdf](http://wwwb.mmm.fi/julkaisut/tyoryhmamuistiot/2003/tr2003_9a.pdf)
- Anonymous. Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys and pigs and *Campylobacter jejuni* and *C. coli* in broilers. *The EFSA Journal*. 2006b, **403**, 1-16. <http://www.efsa.europa.eu>
- Arlet, G., Barret, T. J., Butaye, P., Cloekaert, A., Mulvey, M. R., and White, D. G.: *Salmonella* resistant to extended spectrum cephalosporins: prevalence and epidemiology. *Microbes and Infection*. 2006, **8**, 1945-54

- Asensio, A., Guerrero, A., Quereda, C., Lizan, M., and Martinez-Ferrer, M. Colonisation and infection with methicillin-resistant *Staphylococcus aureus* associated factors and eradication. *Infection Control and Hospital Epidemiology*. 1996, **17**, 20-8
- Batchelor, M., Clifton-Hadley, F. A., Stallwood, A. D., Piba, G. A., Davies, R. H., and Liebana, E. Detection of multiple cephalosporin-resistant *Escherichia coli* from a cattle faecal sample in Great Britain. *Microbial Drug Resistance*. 2005a, **11**, 58-61
- Batchelor, M., Hopkins, K., Threlfall, E. J., Clifton-Hadley, F. A., Stallwood, A. D., Davies, R. H., and Liebana, E. bla<sub>CTX-M</sub> genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. *Antimicrobial Agents and Chemotherapy*. 2005b, **49**, 1319-22
- Batchelor, M., Threlfall, E. J., and Liebana, E. Cephalosporin resistance among animal associated *Enterobacteria*: a current perspective. *Expert Reviews on Anti-Infective Therapy*. 2005c, **3**, 403-17
- Ben-Ami, R., Schwaber, M. J., Navon-Venezia, S., Schwartz, D., Giladi, M., Chmelnitsky, I., Leavitt, A., and Carmeli, Y. Influx of extended -spectrum beta-lactamase-producing *Enterobacteriaceae* into the hospital. *Clinical Infectious Diseases*. 2006, **42**, 925-34
- Berge, A. C. B., Adaska, J. m., and Sischo, W. M. Use of antibiotic susceptibility patterns and pulsed-field gel electrophoresis to compare historic and contemporary isolates of multidrug-resistant *Salmonella enterica*. *Applied and Environmental Microbiology*. 2004, **70**, 318-23
- Berge, A. C., Moore, D. A., and Sischo, W. M. Field trial evaluating the influence of prophylactic and therapeutic antimicrobial administration on antimicrobial resistance of fecal *Escherichia coli* in dairy calves. *Applied and Environmental Microbiology*. 2006, 3872-8
- Bertrand, S., Weill, F.-X., Cloeckaert, A., Vrints, M., Mairaux, E., K., P., Dierick, K., Wildemaue, C., Godard, C., Butaye, P., Imberechts, H., Grimont, P. A., and Collard, J.-M. Clonal emergence of extended-spectrum beta-lactamase (CTX-M-2) producing *Salmonella enterica* serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). *Journal of Clinical Microbiology*. 2006, **44**, 2897-2903
- Biedenbach, D. J., Toleman, M., Walsh, T. R., and Jones, R. N. Analysis of *Salmonella* spp. with resistance to extended-spectrum cephalosporins and fluoroquinolones in North America and Latin America: report from the SENTRY antimicrobial surveillance program (1997-2004). *Diagnostic Microbiology and Infectious Diseases*. 2006, **54**, 13-21
- Blanc, V., Mesa, R., Saco, M., Lavilla, S., Prats, G., Miro, E., Navarro, F., Cortes, P., and Llagostera, M. ESBL- and plasmidic class C betalactamase producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Veterinary Microbiology*. 2006, **118**, 299-304
- Blásquez, J., Morosini, M.-I., Negri, M.-C., and Baquero, F. Selection of naturally occurring extended-spectrum TEM betalactamase variants by fluctuating beta-lactam pressure. *Antimicrobial Agents and Chemotherapy*. 2000, **44**, 2182-4
- Boyce, J., Cookson, B., Christiansen, K., Hori, S., Vuopio-Varkila, J., Kocagöz, S., Öztop, A. Y., Vandenbroucke-Grauls, C. M. J. E., Harbart, S., and Pittet, D. Meticillin-resistant *Staphylococcus aureus*. *The Lancet Infectious Diseases*. 2005, **5**, 653-63

- Bradford, P. A., Petersen, P. J., Fingerman, I. m., and White, D. G. Characterization of expanded spectrum cephalosporin resistance in *E. coli* isolates associated with bovine calf diarrhoeal disease. *Journal of Antimicrobial Chemotherapy*. 1999, **44**, 607-10
- Brinas, L., Moreno, M. A., Teshager, T., Saenz, Y., Porrero, M. C., Domingues, L., and Torres, C. Monitoring and characterization of extended-spectrum beta-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. *Antimicrobial Agents and Chemotherapy*. 2005, **49**, 1262-4
- Brinas, L., Moreno, M. A., Teshager, T., Zarazaga, M., Saenz, Y., Porrero, M. C., Domingues, L., and Torres, C. beta-lactamase characterization in *Escherichia coli* isolates with diminished susceptibility or resistance to extended spectrum cephalosporins recovered from sick animals in Spain. *Antimicrobial Agents and Chemotherapy*. 2003a, **49**, 1262-4
- Brinas, L., Moreno, M. A., Zarazaga, M., Porrero, M. C., Saenz, Y., Garcia, M., Domingues, L., and Torres, C. Detection of CMY-2, CTX-M-14 and SHV-12 beta-lactamases in *Escherichia coli* faecal sample isolates from healthy chickens. *Antimicrobial Agents and Chemotherapy*. 2003b, **47**, 2056-58
- Brinas, L., Zarazaga, M., Saenz, Y., Ruiz-Larrera, F., and Torres, C. beta-lactamase characterization in ampicillin-resistant *Escherichia coli* isolates obtained from foods, humans and healthy animals. *Antimicrobial Agents and Chemotherapy*. 2002, **46**, 3156-63
- Bush, K., Jacoby, G. A., and Medeiros, A. A. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*. 1995, **39**, 1211-33
- Canton, R., and Coque, T. M. The CTX-M beta-lactamase pandemic. *Current Opinion in Microbiology*. 2006, **9**, 466-75
- Carattoli, A. Animal reservoirs for extended spectrum beta-lactamase producers. *Clinical microbiology and infection*, 2008, **14 suppl**, 117-123.
- Carattoli, A., Tosini, F., Giles, W. P., Rupp, M. E., Hinrichs, S. H., Angulo, F., Barrett, T. J., and Fey, P. D. Characterization of plasmids carrying CMY-2 from expanded spectrum cephalosporin-resistant *Salmonella* strains isolated in the United States between 1996 and 1998. *Antimicrobial Agents and Chemotherapy*. 2002, **46**, 1269-1272
- Cavaco L.M., Abatih E, Aarestrup F.M., Guardabassi L. Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. *Antimicrobial Agents Chemotherapy*. 2008, **52**, 3612-6.
- Chaslus-Dancla, E., Pohla, P., Meurisse, M., Marin, M., and Lafont, J. P. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides, gentamicin and apramycin. *Antimicrobial Agents and Chemotherapy*. 1991, **35**, 590-591
- Chen, S., Zhao, S., White, D. G., Schroeder, C. M., Lu, R., Yang, H., McDermott, P. F., Ayers, S., and Meng, J. Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. *Applied and Environmental Microbiology*. 2004, **70**, 1-7
- Cheung, T. K. M., Y.W., C., Chu, M. Y., Choi, H. M., Yung, R. W. H. Y., and Kam, K. M. Plasmid-mediated resistance to ciprofloxacin and cefotaxime in clinical isolates of *Salmonella enterica* serotype enteritidis in Hong Kong. *Journal of Antimicrobial Chemotherapy*. 2005, **56**, 586-89

- Chiu, C. H., Su, L. H., Chia, J. H., Wu, T. L., Lee, Y. S., and Ou, J. T. Isolation of *Salmonella enterica* serotype choleresuis resistant to ceftriaxone and ciprofloxacin. *The Lancet*. 2004, **363**, 1285-86
- Coenen, S., Ferech, M., Dvorakova, K., Hendrickx, E., Suetens, C., and Goossens, H. European surveillance of antimicrobial consumption (ESAC): outpatient cephalosporin use in Europe. *Journal of Antimicrobial Chemotherapy*. 2006, **58**, 401-7
- Corpet, D. E. Antibiotic resistance from food. *New England Journal of Medicine*. 1988, **318**, 1206-7
- DANMAP 2006. Use of antimicrobial agents and occurrence of resistance in bacteria from food animals, foods and humans in Denmark. (<http://www.dfvf.dk>)
- de Neeling, A. J., van den Broek, M. J., Spalburg, E. C., van Santen-Verheuevel, M. G., Dam-Deisz, W. D., Boshuizen, H. C., van de Giessen, A. W., van Duijkeren, E., and Huijsdens, X. W. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Veterinary Microbiology* 2007, **122**, 366-72.
- Deurenberg, R. H., Vink, C., Kelenic, S., Friedrich, A. W., Bruggeman, C. A., and Stobbering, E. E. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection*. 2006, **13**, 222-235
- Devasia, R. A., Varma, J. K., Whichard, J., Gettner, S., Cronquist, A. B., Hurd, S., Segler, S., Smith, K., Hoeffler, D., Shifferaw, B., Angulo, F. J., and Jones, T. F. Antimicrobial use and outcomes in patients with multidrug-resistant and pansusceptible *Salmonella* Newport infections. *Microbial Drug Resistance*. 2005, **11**, 371-77
- Donaldson, S. C., Straley, B. A., Hegde, V., Sawant, A. A., DebRoy, C., and Jayarao, B. M. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. *Applied and Environmental Microbiology*. 2006, **72**, 3940-8
- Dunne, E. F., Fey, P. D., Kludt, P., Reporter, R., Mostashari, F., Shillam, P., Wicklund, J., Miller, C., Holland, B., Stamey, K., Barrett, T. J., Rasheed, J. K., Tenover, F. C., Ribot, E. M., and Angulo, F. J. Emergence of domestically acquired ceftriaxone-resistant salmonella infections associated with AmpC beta-lactamase. *Journal of the American Medical Association*. 2000, **284**, 3151-6
- Erskine, R., Bartlett, P., VanLente, J. L., and Phipps, C. R. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. *Journal of Dairy Science*. 2002, **85**, 2571-5.
- Espie, E., De\_Valk, H., Valliant, V., Quelquejeu, N., Le\_Querrec, F., and Weill, F. X. An outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections linked to the consumption of imported horse meat in France. *Epidemiology and Infection*. 2004, **133**, 373-6.
- Federal Veterinary Association and the Working Group of Chief Veterinary Officers. Guidelines for prudent use of antimicrobial substances in veterinary medicine. *Deutsches Tierärzteblatt (Official German Veterinary Journal)* 2000, **48**, No. 11 Supplement.
- Ferech, M., Coenen, S., Malhotra-Kumar, S., Dvorakova, K., Hendrickx, E., Suetens, C., and Goossens, H. European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in Europe. *Journal of Antimicrobial Chemotherapy*. 2006, **58**, 401-7.
- Fey, P. D., Safraneck, T. J., Rupp, M. E., Dunne, E. F., Ribot, E., Iwen, P. C., Bradford, P. A., Angulo, F. J., and Hinrichs, S. H. Ceftriaxone-resistant salmonella infection acquired by a child from cattle [see comments]. *New England Journal of Medicine*. 2000, **342**, 1242-9.

- FINRES-vet 2005-2006*. Finnish veterinary antimicrobial resistance monitoring and consumption of antimicrobial agents. Evira publications 22/2007. <http://www.evira.fi>
- Frye, J. G., and Fedorka-Cray, P. J. Prevalence, distribution and characterisation of ceftiofur resistance in *Salmonella enterica* isolated from animals in the USA from 1999 to 2003. *International Journal of Antimicrobial Agents*. 2007, **30**, 134-42
- FVE (1999) Antibiotic resistance & prudent use of antibiotics in veterinary medicine, Federation of veterinarians of Europe.  
[http://www.fve.org/papers/pdf/vetmed/position\\_papers/antibioen.pdf](http://www.fve.org/papers/pdf/vetmed/position_papers/antibioen.pdf)
- Giguère, S. Antimicrobial drug use in horses. In: Giguère, S., Prescott, J.F., Baggot, J.D., Walker, R.D., Dowling, P.M (eds). *Antimicrobial therapy in veterinary medicine*, 4<sup>th</sup> ed. 2006, Blackwell publishing Ltd ISBN-13:978-0-8138-0656-3. p. 449-462.
- Girlich, D., Poirel, L., Carattoli, A., Kempf, I., Lartigue, M.-F., Bertini, A., and Nordmann, P. Extended-Spectrum beta-lactamase CTX-M-1 in *Escherichia coli* in healthy poultry in France. *Applied Environmental Microbiology*. 2007, **73**, 4681-5.
- Gniadkowski M. Evolution of extended-spectrum beta-lactamases by mutation. *Clinical Microbiology and Infection*. 2008, 14 Suppl **1**, 11-32.
- Gniadkowski, M. Evolution and epidemiology of extended-spectrum beta-lactamase (ESBLs) and ESBL-producing organisms. *Clinical Microbiology and Infection*. 2001, **7**, 597-608.
- Gootz, T. D. Global dissemination of beta-lactamases mediating resistance to cephalosporins and carbapenems. *Expert Reviews on Anti-infective Therapy*. 2004, **2**, 317-327.
- Gray, J. T., Hungerford, L. L., Fedorka-Key, P. J., and Headrick, M. L. Extended-spectrum-cephalosporin resistance in *Salmonella enterica* isolates of animal origin. *Antimicrobial Agents and Chemotherapy*. 2004, **48**, 3179-81.
- Guardabassi, L., Stegger, M., and Skov, R. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus* ST398 in Danish slaughter pigs. *Veterinary Microbiology*. 2007, **122**, 384-6.
- Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S., Hekstra, M. P., Whichard, J. M., Barrett, T. J., and Angulo, F. J. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to extended spectrum cephalosporins in the United States. *Journal of Infectious Diseases*. 2003, **188**, 1707-16.
- Hasman, H., Mevius, D., Veldman, K., Olesen, I., and Aarestrup, F. M. beta-lactamases among extended spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *Journal of Antimicrobial Chemotherapy*. 2005, **56**, 115-21.
- Hopkins, K. L., Liebana, E., Villa, L., Batchelor, M., Threlfall, E. J., and Carattoli, A. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. *Antimicrob Agents Chemother*. 2006, **50**, 3203-6.
- Hornish, R. E., and Kotarski, S. F. Cephalosporins in veterinary medicine - ceftiofur use in food animals. *Current Topics in Medicinal Chemistry*. 2002, **2**, 717-31.
- Horton, J. M., Sing, R. F., and Jenkins, S. G. Multidrug-resistant *Salmonella* associated with AmpC hyperproduction. *Clinical Infectious Diseases*. 1999, **29**, 1348.

- Hunter, J. E. B., Bennett, M., Hart, C. A., Shelley, J. C., and Walton, J. R. Apramycin-resistant *Escherichia coli* isolated from pigs and a stockman. *Epidemiology and Infection*. 1994, **112**, 473-80.
- Jacoby, G. A., and Munoz-Price, L. S. The new beta-lactamases. *New England Journal of Medicine*. 2005, **352**, 380-91.
- Jacoby, G. A. beta-lactamase nomenclature. *Antimicrobial Agents and Chemotherapy*. 2006, **50**, 1123-29.
- Jorgensen, C. J., Cavaco, L. M., Hasman, H., Emborg, H.-D., and Guardabassi, L. Occurrence of CTX-M-1-producing *Escherichia coli* in pigs with ceftiofur. *Journal of Antimicrobial Chemotherapy*. 2007, **59**, 1040-2.
- Kahlmeter G. Breakpoints for intravenously used cephalosporins in *Enterobacteriaceae*—EUCAST and CLSI breakpoints. *Clinical Microbiology and Infection*. 2008. **14**, Suppl 1:169-74.
- KNMvD. De formularia. Richtlijnen voor verantwoord antibioticumgebruik. Koninklijke Nederlandse Maatschappij voor Diergeneeskunde. 2007.  
[http://www.knmvd.nl/resources/AMGATE\\_7364\\_1\\_TICH\\_R2361006779334/](http://www.knmvd.nl/resources/AMGATE_7364_1_TICH_R2361006779334/)
- Ko, W. C., Yan, J. J., Yu, W. L., Lee, H. C., Lee, N. Y., Wang, L. R., and Chuang, Y. C. A new therapeutic challenge for old pathogens: community acquired invasive infections caused by ceftriaxone- and ciprofloxacin resistance *Salmonella enterica* serotype Choleraesuis. *Clinical Infectious Diseases*. 2005, **40**, 315-8
- Koeck, J. L., Arlet, G., Philippon, A., Basnaciogullari, H., Vu\_Thien, H., Buisson, Y., and Cavallo, J. D. A plamid-mediated CMY-2 beta-lactamase from an Algerian clinical isolate of *Salmonella senftenberg*. *FEMS Microbiology Letters*. 1997, **152**, 255-60.
- Kojima, A., Ishii, K., Esaki, H., Asai, T., Oda, C., Tamura, Y., Takahashi, T., and Yamaguchi, K. Extended-spectrum-beta-lactamase producing *Escherichia coli* strains isolated from farm animals from 1999 to 2002: report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Antimicrobial Agents and Chemotherapy*. 2005, **49**, 3533-7.
- Kruse, H., and Sørum, H. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied Environmental Microbiology*. 1994, **60**, 4015-21.
- Leonard, F. C., and Markey, B. K. Meticillin resistant *Staphylococcus aureus* in animals: a review. *Veterinary Journal*. 2008, **175**, 27-36.
- Lester, C. H., Moller, N.-F., Sorensen, T. L., Monnet, D. L., and Hammerum, A. M. In vivo transfer of the *vanA* resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. *Antimicrobial Agents and Chemotherapy*. 2006, **50**, 596-9
- Le Turnier S., Nordmann P., Eb F., Mammeri H. Potential evolution of hydrolysis spectrum for AmpC  $\beta$ -lactamase of *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*. 2009, **63**, 216-8.
- Levy, S. B., FitzGerald, G. B., and Macone, A. B. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. *Nature*. 1976, **260**, 40-2

- Li, X. Z., Mehrotra, M., Ghimire, S., and Adewoye, L. beta-Lactam resistance and beta-lactamases in bacteria of animal origin. *Veterinary Microbiology*. 2007, **121**, 197-214.
- Liebana, E., Batchelor, M., Hopkins, K. L., Clifton-Hadley, F. A., Teale, C. J., Foster, A., Barker, L., Therlfall, E. J., and Davies, R. H. Longitudinal farm study of extended-spectrum beta-lactamase-mediated resistance. *Journal of Clinical Microbiology*. 2006, **44**, 1630-4
- Liebana, E., Gibbs, M., Clouthing, C., Barker, L., Clifton-Hadley, F. A., Pleydell, E., Abdalhamid, B., Hanson, N. D., Martin, L., Poppe, C., and Davies, R. Characterization of beta-lactamases responsible for resistance to extended spectrum cephalosporins in *Escherichia coli* and *Salmonella enterica* strains from food-producing animals in the United Kingdom. *Microbial Drug Resistance*. 2004, **10**, 1-9.
- Livermore, D. M., and Brown, D. F. J. Detection of beta-lactamase mediated resistance. *Journal of Antimicrobial Chemotherapy*. 2001, **48**, 59-64.
- Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G., Arlet, G., Ayala, J., Coque, T. M., Kern-Zdanowicz, I., Luzzaro, F., Poirel, L., and Woodford, N. CTX-M: changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy*. 2007, **59**, 165-74.
- Lopes, V. C., Wedel, S. D., Bender, J. B., Smith, K. E., Leano, F. T., Boxrud, D. J., Lauer, D. C., Velayudhan, B. T., and Nagarja, K. V. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport in Minnesota. *Clinical Infectious Diseases*. 2006, **43**, 210-3.
- Lowrance, T. C., Loneragan, G. H., Kunze, D. J., Platt, T. M., Ives, S. E., Scott, H. M., Norby, B., Echeverry, A., and Brashears, M. M. Changes in antimicrobial susceptibility in a population of *Escherichia coli* isolated from feedlot cattle administered ceftiofur crystalline-free acid. *American Journal of Veterinary Research*. 2007, **68**, 501-7.
- Machado E, Coque TM, Cantón R, Sousa JC, Peixe L. Antibiotic resistance integrons and extended-spectrum {beta}-lactamases among *Enterobacteriaceae* isolates recovered from chickens and swine in Portugal. *Journal of Antimicrobial Chemotherapy*. 2008, **62**, 296-302.
- Mammeri H, Eb F, Berkani A, Nordmann P. Molecular characterization of AmpC-producing *Escherichia coli* clinical isolates recovered in a French hospital. *Journal of Antimicrobial Chemotherapy*. 2008, **61**, 498-503.
- Mammeri H, Poirel L, Nordmann P. Extension of the hydrolysis spectrum of AmpC beta-lactamase of *Escherichia coli* due to amino acid insertion in the H-10 helix. *Journal of Antimicrobial Chemotherapy*. 2007, **60**, 490-4.
- MARAN (2005) Monitoring of antimicrobial resistance and antibiotic usage in the Netherlands in 2004. <http://www.cidc-lelystad.nl>
- Medeiros, A. A. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clinical Infectious Diseases*. 1997, **24(suppl 1)**, S19-45.
- Meunier, D., Jouy, E., Iazzera, C., Kobisch, M., and Madec, J.-Y. CTX-M-1- and CTX-M-15-type beta-lactamases in clinical *Escherichia coli* isolates recovered from food-producing animals in France. *International Journal of Antimicrobial Agents*. 2006, **28**, 402-7.
- Michael, G. B., Butaye, P., Cloeckert, A., and Schwartz, S. Genes and mutations conferring antimicrobial resistance in *Salmonella*: and update. *Microbes and Infection*. 2006, **8**, 1898-914.

- Mirelis B, Navarro F, Miró E, Mesa RJ, Coll P, Prats G. Community transmission of extended-spectrum beta-lactamase. *Emerging Infectious Diseases*. 2003 **9**, 1024-5.
- Miriagou, V., Tasios, P. T., Legakis, N. J., and Tzouvelekis, L. S. Expanded-spectrum cephalosporin resistance in non-typhoid *Salmonella*. *International Journal of Antimicrobial Agents*. 2004, **23**, 547-55.
- Moreno, M. A., Teschager, A., Porreno, C. M., Garcia, M., Escudero, E., Torres, C., and Domiguez, L. Abundance and phenotypic diversity of *Escherichia coli* isolates with diminished susceptibility to expanded-spectrum cephalosporins in faeces from healthy food animals after slaughter. *Veterinary Microbiology*. 2007, **120**, 363-9.
- Mulvey M.R, Susky E, McCracken M., Morck D.W., Read R.R. Similar cefoxitin-resistance plasmids circulating in *Escherichia coli* from human and animal sources. *Veterinary Microbiology*. 2008.
- Nikolich, M. P., Hong, G., Shoemaker, N. B., and Salyers, A. A. Evidence for natural horizontal transfer of *tetQ* between bacteria that normally colonize humans and bacteria that normally colonize livestock. *Applied and Environmental Microbiology*. 1994, **60**, 3255-60
- Passantino, A. Ethical aspects for veterinarians regarding antimicrobial drug use in Italy. *International Journal of Antimicrobial Agents*. 2007, **29**, 240-4
- Paterson, D. L., and Bonomo, R. A. Extended spectrum beta-lactamases: a clinical update. *Clinical Microbiology Reviews*. 2005, **18**, 657-86
- Pfaller, M. A., and Segreti, J. Overview of the epidemiological profile and laboratory detection of extended spectrum beta-lactamases. *Clinical Infectious Diseases*. 2006, **42**, S153-63
- Pitout, J. D. D., Reisbig, M. D., Mulvey, M., Chui, L., Loue, M., Crowe, L., Church, D. L., Elsayed, S., Gregson, D., Ahmed, R., Tilley, P., and Hanson, N. D. Association between handling of pet treats and infection with *Salmonella enterica* serotype Newport expressing the AmpC beta-lactamase, CMY-2. *Journal of Clinical Microbiology*. 2003, **41**, 4578-82
- Politi, L., Tassios, P. T., Lambiri, M., Kansouzidou, A., Pasiotou, M., Vatopoulos, A. C., Mellou, K., Legakis, N. J., and Tzouvelekis, L. S. Repeated occurrence of diverse extended-spectrum beta-lactamases in minor serotypes of food-borne *Salmonella enterica* subsp *enterica*. *Journal of Clinical Microbiology*. 2005, **43**, 3453-6
- Pomba C., Coelho Baptista B, Diniz da Fonseca J, Duarte Cor J. First description of CTX-M-1- and CMY-2-producing *Escherichia coli* isolates recovered from coli-septicemic broilers in Portugal (A45). Presented at: ASM conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, June 15-17 2008. Copenhagen, Denmark.
- Poutrel B., Stegemann MR., Roy O., Pothier F., Tilt N., and Payne-Johnson M. Evaluation of the efficacy of systemic danofloxacin in the treatment of induced acute *Escherichia coli* bovine mastitis. *Journal of Dairy Research*. 2008. **75**, 310-8.
- Poppe, C., Martin, L. C., Reid-Smith, R., Boerlin, P., McEwen, S. A., Prescott, J. F., and Forward, K. R. Acquisition of resistance to extended-spectrum cephalosporins by *Salmonella enterica* serovar Newport and *Escherichia coli* in the turkey poult intestinal tract. *Applied and Environmental Microbiology*. 2005, **71**, 1184-92
- Quale, J., Landman, D. M., Bradford, P. A., Visalli, M., Ravishankar, J., Flores, C., Mayorga, D., Vangala, K., and Adedeji, A. Molecular epidemiology of a city-wide outbreak of

extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* infection. *Clinical Infectious Diseases*. 2002, **35**, 834-41.

Queenan, A. M., Foleno, B., Gownley, C., Wira, E., and Bush, K. Effects of inoculum and beta-lactamase activity in AmpC- and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. *Journal of Clinical Microbiology*. 2004, **42**, 269-75.

Rantala, M., Kaartinen, L., Välimäki, E., Stryman, M., Hiekkaranta, M., Niemi, A., Saari, L., Pyörälä, S. Efficacy and pharmacokinetics of enrofloxacin and flunixin meglumine for treatment of cows with experimentally induced *Escherichia coli* mastitis. *Journal of Veterinary Pharmacology and Therapeutics*. 2002 **25**, 251-8.

Riano, I., Moreno, M. A., Teshager, T., Saenz, Y., Dominguez, L., and Torres, C. Detection and characterization of extended-spectrum beta-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. *Journal of Antimicrobial Chemotherapy*. 2006, **58**, 844-7.

Robicsek, A., Jacoby, G. A., and Hooper, D. C. The worldwide emergence of plasmid mediated quinolone resistance. *The Lancet Infectious Diseases*. 2006a, **6**, 629-40.

Robicsek, A., Strahilevitz, J., Jacoby, G. A., Macielag, M., Abbanat, D., Park, C. H., Bush, K., and Hooper D. C. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nature Medicine*. 2006b, **12**, 83-8.

Rodriguez-Bano, J. & Navarro, M.D. Extended spectrum beta-lactamases in ambulatory care: a clinical perspective. *Clinical Microbiology and Infection*. 2008, **14**, suppl 1 104-10.

Sampimon, O.C., Olde Riekerink, R.G.M., Lam, T.J.G.M. Prevalence of subclinical mastitis pathogens and adoption of udder health management practices on Dutch dairy farms: preliminary results. *Mastitis Control: from Science to Practice*. Haag, The Netherlands, September 30-October 2, 2008. Proceedings, p. 39-46.

Saurina, G., Quale, J. M., Manikal, V. M., Qydna, E., and Landman, D. Antimicrobial resistance in Enterobacteriaceae in Brooklyn, N.Y.: epidemiology and relation to antibiotic usage patterns. *Journal of Antimicrobial Chemotherapy*. 2000, **45**, 895-98.

Schwaber, M., and Carmeli, Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteremia: a systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy*. 2007, **60**, 913-20.

Shiraki, Y., Shibata, N., Doi, Y., and Arakawa, Y. *Escherichia coli* producing CTX-M-2 beta-lactamase in cattle in Japan. *Emerging infectious diseases*. 2004, **10**, 69-75.

Shpigel, N., Levin, D., Winkler, M., Saran, A., Ziv, G., and Bottner, A. Efficacy of cefquinome for treatment of cows with mastitis experimentally induced using *Escherichia coli*. *Journal of Dairy Science*. 1997, **80**, 318-23.

Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, Herman L, Haesebrouck F, Butaye P. Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* Isolates in Belgian broiler farms. *Antimicrobial Agents Chemotherapy*. 2008, **52**, 1238-43.

Su, L.-H., Chiu, C.-H., Chu, C., Wang, M.-H., Chia, J.-H., and Wu, T.-L. In vivo acquisition of ceftriaxone resistance in *Salmonella enterica* serotype anatum. *Antimicrobial Agents and Chemotherapy*. 2003, 563-7.

- SVARM. 2006. Swedish Veterinary Antimicrobial Resistance Monitoring 2006. The National Veterinary Institute (SVA), Uppsala, Sweden. <http://www.sva.se>.
- Tacconelli E, De Angelis G, Cataldo MA, Pozzi E, Cauda R. Does antibiotic exposure increase the risk of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation? A systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy*. 2008, **61**, 26-38.
- Teale, C. J., Barker, A. P., Foster, E., Liebana, E., Batchelor, M., Livermore, D. M., and Therlfall, E. J. Extended spectrum beta-lactamases detected in *E. coli* recovered from calves in Wales. *Veterinary Record*. 2005, **156**, 186-7.
- Tragesser, L. A., Wittum, T. E., Funk, J. A., Winokur, P. L., and Rajala-Schultz, P. J. Association between ceftiofur use and isolation of *Escherichia coli* with reduced susceptibility to ceftriaxone from fecal samples of dairy cows. *American Journal of Veterinary Research*. 2006, **67**, 1696-1700.
- Tschäpe, H. The spread of plasmids as a function of bacterial adaptability. *FEMS Microbiology letters*. 1994, **15**, 23-32.
- Urbanek, K., Kolar, M., Loveckova, Y., Strojil, J., and Santava, L. Influence of third-generation cephalosporin utilization on the occurrence of ESBL-positive *Klebsiella pneumoniae* strains. *Journal of Clinical Pharmacology and Therapeutics*. 2007, **32**, 403-8.
- Valverde A, Coque TM, Sánchez-Moreno MP, Rollán A, Baquero F, Cantón R. Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* during nonoutbreak situations in Spain. *Journal of Clinical Microbiology*. 2004 **42**, 4769-75.
- Vander Stichele, R. H., Elseviers, M. M., Ferech, M., Blot, S., and Goossens, H. Hospital consumption of antibiotics in 15 European countries: results of the ESAC retrospective data collection (1997-2002). *Journal of Antimicrobial Chemotherapy*. 2006, **58**, 159-67.
- Vo, A. T. T., van Duijkeren, E., Fluit, A. C., and Gaastra, W. Characteristics of extended spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from horses. *Veterinary Microbiology*. 2007, **124**, 248-55.
- Wachino J, Kurokawa H, Suzuki S, Yamane K, Shibata N, Kimura K, Ike Y, Arakawa Y. Horizontal transfer of blaCMY-bearing plasmids among clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates and emergence of cefepime-hydrolyzing CMY-19. *Antimicrobial Agents and Chemotherapy*. 2006, **50**, 534-41.
- Weese, J.S., Baptiste, K.E., Baverud, V., Toutain, P.L. Guidelines for antimicrobial use in horses. In: Guardabassi, L., Jensen, L.B., Kruse, H, /eds) *Guide to antimicrobial use in animals*. 2008. Blackwell publishing ltd. ISBN-13: 978-1-4051-5079-9. p. 161-182.
- Weill, F. X., Lailier, R., Praud, K., Kerouanton, A., Fabre, L., Brisabois, A., Grimont, P. A. D., and Clockaert, A. Emergence of extended-spectrum-beta-lactamase (CTX-M-9)-producing multiresistant strains in *Salmonella enterica* serotype Virchow in poultry and humans in France. *Journal of Clinical Microbiology*. 2004, **42**, 5767-73.
- White, D. G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P. F., Wagner, D. D., and Meng, J. The isolation of antibiotic-resistant salmonella from retail ground meat. *New England Journal of Medicine*. 2001, **345**, 1147-1154.
- Winokur, P. L., Brueggemann, A., DeSalvo, D. L., Hoffmann, L., Apley, M. D., Uhlenhopp, E. K., Pfaller, M. A., and Doern, G. V. Animal and human multidrug-resistant, cephalosporin-

resistant salmonella isolates expressing a plasmid-mediated CMY-2 AmpC beta-lactamase. *Antimicrobial Agents and Chemotherapy*. 2000, **44**, 2777-83

Winokur, P. L., Vonstein, D. L., Hoffmann, L., Uhlenhopp, E. K., and Doern, G. V. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. *Antimicrobial Agents and Chemotherapy*. 2001, **45**, 2716-22

Witte, W., Strommenger, B., Stanek, C., and Cuny, C. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. *Emerging Infectious Diseases*. 2007, **13**, 255-8.

Yan, J.-J., Chiou, C.-S., Lauderdale, T.-L. Y., Tsai, S.-T., and Wu, J.-J. Cephalosporin and ciprofloxacin resistance in *Salmonella*, Taiwan. *Emerging Infectious Diseases*. 2005, **11**, 947-50

Zhao, S., Qaiyumi, S., Friedman, S., Singh, R., Foley, S. L., White, D. G., McDermott, P. F., Donkar, T., Bolin, C., Munro, S., Baron, E. J., and Walker, R. D. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *Journal of Clinical Microbiology*. 2003, **41**, 5366-71

Zhao, S., White, D. G., McDermott, P. F., Friedman, S., English, L., Ayers, S., Meng, J., Maurer, J. J., Holand, R., and Walker, R. D. Isolation and expression of cephamycinase bla<sub>CMY</sub> genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. *Antimicrobial Agents and Chemotherapy*. 2001, **45**, 3647-50