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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE (CVMP)

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

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The CVMP Scientific Advisory Group on Antimicrobials (SAGAM) has provided the scientific information included in the document.

The list of Risk Management (recommendations for action) measures has been prepared by CVMP.

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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 3rd and 4th generation cephalosporins.

This document discusses cephalosporins with a focus on substances of the 3rd and 4th generation and food production animals, excluding aquaculture.

INTRODUCTION

Cephalosporins of the 3rd and 4th generation represent subclasses of antimicrobials which are very important in the treatment of severe and invasive infections in humans and 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 80s (Gniadkowski, 2001). Since then, the occurrence of infections with bacteria resistant to 3rd and 4th 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 3rd and 4th 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 3rd and 4th 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 also e.g. *E. coli* and *Salmonella* (Anonymous, 2006; 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 behaviour, 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 (Livermore *et al*., 2007). This suggests that animals may act as possible important reservoirs for transferable beta-lactamases. Third and 4th 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 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 3rd and 4th 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.

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.

Traditionally, 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. In this document, 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 (Anon., 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. cephaclor, cefoxitin[a], 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[a]) generally have a broad spectrum of activity, with increased stability to many of the beta-lactamases that inactivate the earlier generations 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, 3rd and 4th generation cephalosporins are used for, 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 3rd and 4th generation cephalosporins ranged between 10 and 50%. An increase in the use of 3rd and 4th generation cephalosporins between the years 1997 and 2002 was noted for all countries.

[a] a cephamycin

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 1st and 2nd generation cephalosporins. Cephalosporins of higher generations have mainly been available for injection or infusion, and their use has probably mostly been limited to patients in e.g. elderly care with severe community acquired pneumonia or complicated urinary tract infections. However, some products for oral use have been introduced on the market. In three countries, use of 3rd and 4th 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 3rd 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 of antimicrobials (Vander Stichele *et al*., 2006). However, 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, cephalexin 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.

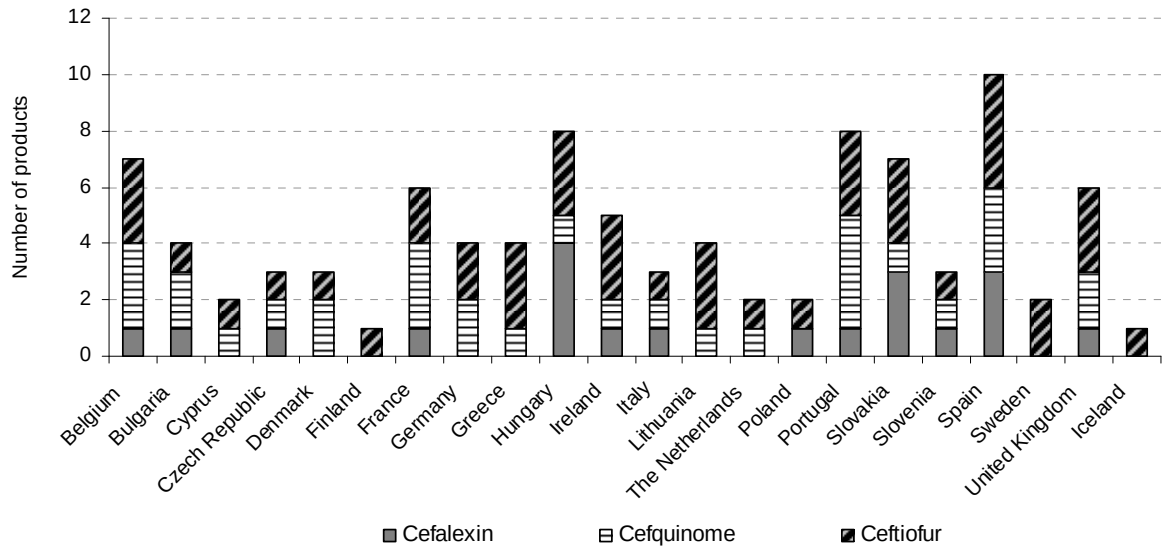

Figure 1. Number of products formulated for injection per antimicrobial substance and Member State.

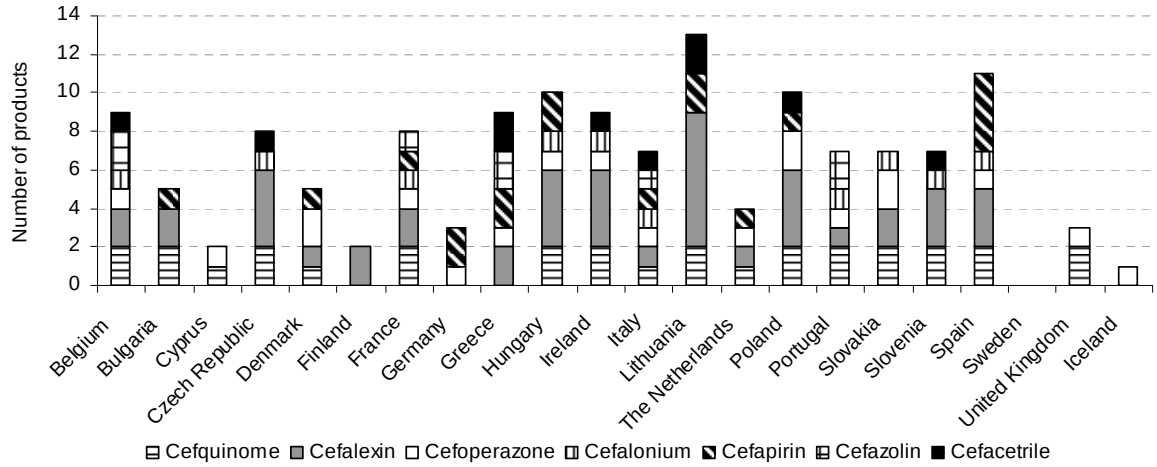

Figure 2. Number of products formulated for intramammary use per antimicrobial substance and Member State.

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 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, and in particular use for groups or flocks of animals, is likely to have the major impact. Potential effects of intramammary use (i.e. topical use) does not share these characteristics and will therefore not be further addressed in this document. Of the substances authorised for systemic use, cephalexin is a 1st, ceftiofur a 3rd and cefquinome a 4$^{th.}$ generation cephalosporin.

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 has previously been authorised for injection of day-old chickens for prevention of septicaemia (Bertrand *et al*., 2006).

Cefquinome (4th generation) is available in some Member States for systemic use in cattle, pigs and horses. The indications for use of cefquinome are mainly respiratory infections, interdigital necrobacillosis ("foot rot") 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* and mastitis-metritis-agalactia (MMA) syndrome in sows are also included. As 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 situation 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 currently 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 3rd and 4th generation cephalosporins. In the reports on use of antimicrobials for from Denmark and France, data are given per animal species. The amounts for systemic use for pigs were 98 and 1310 kg active substance, respectively. For both countries, this represents 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)[1].

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

[1] 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. However, 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 make these drugs a convenient and attractive choice in many situations.

Currently there is no harmonised approach on prudent use of cephalosporins in animals in the 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 (e.g. Passantino, 2007) but most are on a general level and cephalosporins are not specifically mentioned.

Some national guidelines however give specific recommendations also 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 (Anon., 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 an marketing authorization or a special licence. Off-label use of these products is prohibited.

However, 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). In spite of that, off-label use for non-authorised 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 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 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 resistance (Li *et al*., 2007). The gene encoding this mechanism, *mecA,* is chromosomally located as part of the Staphylococal Cassette Chromosome (SCCmec). SSCmec is horizontally transferable between staphylococci and is commonly present in some species of coagulase negative *Staphylococcus* spp. present in humans and in animals. Methicillin resistant *Staphylococcus aureus* (MRSA) have probably acquired the SCCmec element from coagulase negative staphylococci (Deurenberg *et al*., 2006).

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 hundreds of variants of beta-lactamases have been distinguished. The enzymes are classified according to different schemes. The most commonly used are the schemes 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).

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

Bacterial species	**Beta-lactamase**	**Examples**	**Substrate specificity/Activity pattern**	**Susceptible to clavulanic acid[a]**
S. aureus	Penicillinase	PC-1	Benzylpenicillin, aminopenicillins	++++
Enterobacteriaceae, Pseudomonas and *Acinetobacter*	Broad-spectrum	TEM-1, TEM-2, SHV-1	Benzylpenicillin, amino-penicillin, ureido-penicillins, carboxy-penicillins, 1st 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 plus cephamycins (eg cefotetan, cefoxitin)	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	+

257 [a] The number of plus-signs denote relative susceptibility of the families to inhibitors. Note that within the
258 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 e.g. 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 in turn are 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).

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 fluoroquinolone 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 fluoroquinolones such as ciprofloxacin (Robicsek *et al*., 2006b)

As 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, the low epidemiological cut-off values set by EUCAST are more sensitive than the, mostly higher, clinical break-points set by, e.g. Clinical Laboratory Standards Institute (CLSI). 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 (Anon., 2006b).

RESISTANCE IN BACTERIA FROM FOOD PROCUCING 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 also in food-producing animals (for a review see Leonard & Markey, 2007). 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 Austria, Belgium, Denmark and Germany (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 indicates that it has emerged in animals, probably though transfer of the SCCmec cassette from coagulase negative staphylococci to *S. aureus* and possibly under selection by, e.g., use of cephalosporins.

Resistance in *Enterobacteriaceae*

In Table 3 and 4, phenotypic data on resistance to 3rd generation cephalosporins in *E. coli* from healthy animals and in *Salmonella* have been compiled from the summary report on zoonoses in the EU (Anon., 2006a). 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) indicate that in year 2005, the overall average figure of resistance to cefotaxime in *S.* Typhimurium isolated from humans in EU was 0.6% (Anon., 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), the clinical break-points of the CLSI have been used for interpretation and this may have lead to an underestimation of microbiological resistance (Queenan *et al*., 2004; Tenover *et al*., 2003).

Table 3. Reported resistance to third generation cephalosporins in *Escherichia coli* isolated in healthy animals or food products (percent resistant isolates)[a] (Source: The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005) (Anon., 2006a).

Country reporting	Cattle		Fowl (Gallus gallus)		Pigs		Minced, mixed meat		Meat from cattle		Meat from broilers	
	N[b]	Res (%)[c]	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)
Austria	284	0%	128	0%	226	0%						
Belgium									238	2%	148	3%
Denmark	101	0%	132	0%	136	0%						
Estonia	49	0%			40	0%						
Finland			380	0%								
Germany					30	0%	50	0%				
Greece	50	0%										
Italy	368	0.5%	121	0%	73	0%						
The Netherlands	139	0%	304	14%	299	0.3%						
Norway	98	0%							90	0%		
Poland	220	0.5%	73	0%	342	0.9%						
Spain			74	23%	192	0.5%						
Sweden					390	0%						
United Kingdom			47	0%	125	0%						

[a.] Data taken from 'level 3' annex of the report; only entries with at least 30 isolates tested are included. Tabulated results are those given for 3rd generation cephalosporins (unspecified), cefotaxime, ceftazidime or ceftiofur. For Poland, data on cefuroxim (2nd generation) is given. Data from Slovakia were excluded because of unclarities; [b] Number of isolates tested; [c] Percent of tested isolates reported as resistant.

Table 4. Reported resistance to third generation cephalosporins in *Salmonella* from animals or food products (percent resistant isolates)[a] (Source: The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005).(Anon., 2006a)

Country reporting	*Salmonella* Enteritidis		*Salmonella* Typhimurium						*Salmonella* spp											
	Fowl (Gallus gallus)		Cattle		Fowl (Gallus gallus)		Pigs		Cattle		Fowl (Gallus gallus)		Pigs		Meat from poultry		Meat from cattle		Meat from pigs	
	N[b]	Res (%)[c]	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)	N	Res (%)
Austria	406	0%			48	0%					709	0%			192	0%				
Denmark							737	0%												
Germany	385[d]	0%					302	0%	278	0%	589[d]	0%	414	0%	148	1%	32	0%	568	0%
Italy	67	0%	35	6%	37	0%	55	5%	62	3%	480	0.4%	268	1%	310	3%	34	0%	349	4%
The Netherlands	43[d]	0%					85	0%	90	0%	237	2%	120	0%						
Slovakia	98	0%									126	0%								
Spain							40	5%					132	2%						
United Kingdom	46	0%	71	0%			317	0%	499	0%	778	0%	398	0%						

[a] Data taken from 'level 3' annex of the report; only entries with at least 30 isolates tested are included. Tabulated results are those given for 3rd generation cephalosporins (unspecified), cefotaxime, ceftazidime or ceftiofur; [b] Number of isolates tested; [c] Percent of tested isolates reported as resistant; [d] figure reported for laying hens

As can be seen in Tables 3 and 4, some countries report a high prevalence of resistance to 3rd generation cephalosporins for *E. coli* from poultry. Individual countries also report increasing resistance in *Salmonella* from different animal sources. As an example of a strikingly rapid emergence, data on resistance to cefotaxime in *E. coli* from healthy broilers, and in *Salmonella* Paratyphi B var. Java from broilers in the Netherlands is shown in Figure 3 (data from MARAN 2005 and Dik Mevius personal communication, 2007).

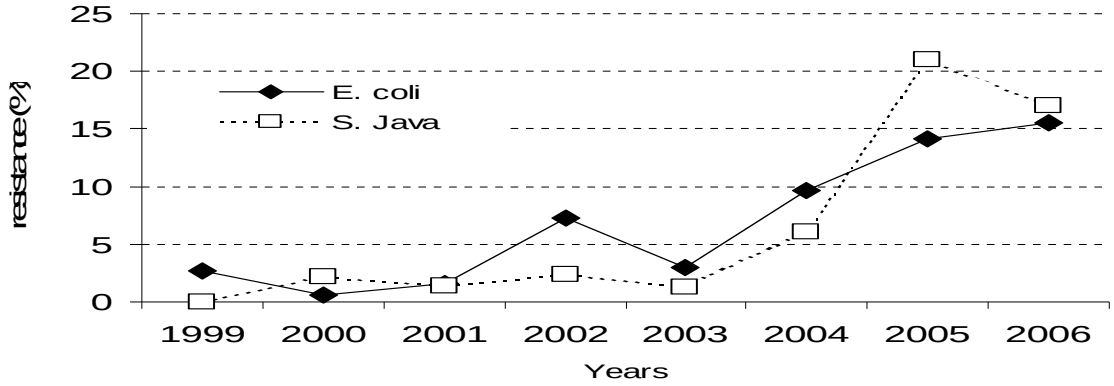

Figure 3. Resistance to cefotaxime in *E. coli* and *Salmonella* Paratyphi B var. Java from broilers in the Netherlands.

The resistance data in Tables 2 and 3 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 3rd 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 very high, only a low percentage of *E. coli* in each sample displayed this trait.

Considering that laboratory detection of these types of resistance can be problematic and underreporting may have occurred, the information above indicates that the resistance to 3rd and 4th generation cephalosporins in *E. coli* and *Salmonella* isolated from animals in Europe is rapidly emerging.

Emergence of ESBLs

Non-typhoidal *Salmonella* spp with ESBL-type resistance appeared in the late 80s-early 90s. Since, 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 seem to be the most commonly encountered (Arlet *et al*., 2006). This is true also 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 reported 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.* Riessen from a pig (Riano *et al*., 2006). From Greece, multiresistant *Salmonella* Virchow producing CTX-M-32 was 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). Further, in a screening using selective techniques, 12 of 112 healthy poultry sampled at slaughter carried CTX-M-1 producing *E. coli* (Girlich *et al*., 2007). 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. In particular, the 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 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 of these 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 predominantely 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 so far only two reports from Europe on production of CMY-2 in *Salmonella* isolated 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, AAC-1, was described in *Salmonella* spp in a study from the Netherlands (Hasman *et al*., 2005). The gene encoding AAC-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 also 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, as much as 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 resistant to tetracycline, sulfonamides and streptomycin, almost 70% to gentamicin and 15% to ciprofloxacin (resistance defined by clinical break-points).

Plasmid mediated AmpC-type beta-lactamases has been reported from Spain at low frequency in *E. coli* isolated from diagnostic submissions from cattle and pigs (Brinas *et al*., 2003a) and 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* 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, the occurrence still seems to be more limited. 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 and only 15 and 5%, of the dose, respectively, is excreted in faeces (EMEA/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 has not been possible to retrieve from publicly available sources. Such information is essential to evaluate the exposure of the gastro-intestinal flora of the target animal to the parent drug or active metabolites (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 can provide a selective pressure, but in particular those substances that resist the action of staphylococcal penicillinase (penicillinase stable penicillins, penicillins in combination with clavulanic acid and all cephalosporins). In human medicine, use of cephalosporins or fluoroquinolones is associated with an increased risk of MRSA colonisation (Asensio *et al*., 1996; Hill *et al*., 1998). 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 flora can be very long. However, as this document is focused on resistance with particular relevance for the 3rd and 4th generation cephalosporins rather than all cephalosporins and other penicillinase stabile beta-lactams, emphasis will 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 to expanded spectrum beta-lactams, but mutations in the genes encoding these enzymes lead to structural changes that can extend or alter the substrate specificity (Gniadkowski, 2001). The evolution of ESBLs has been attributed to the selective pressure exerted by use of third generation cephalosporins (Medeiros, 1997). There are a number of studies in human clinical settings in support of that (Gniadkowski, 2001). Blásquez 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 would explain why many of the enzymes generated *in vitro* never occur naturally – only ESBLs with a truly broad-based resistance will 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 is based on laboratory studies and studies in human clinical settings. It is probable that the general principle applies also to animal production, and that use of 3rd and 4th generation cephalosporins in animal populations, and possibly also of other beta-lactams, is likely to 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 3rd generation cephalosporins is a recognised risk factor for ESBL colonisation of patients in human hospitals (e.g. 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 of plasmid mediated AmpC-type beta-lactamases in *Salmonella* in North America (Allen & Poppe, 2002; Dunne *et al*., 2000; Fey *et al*., 2000; White *et al*., 2001; Winokur *et al*., 2000). However, until recently there have been no specific studies on the influence of the use of 3rd generation cephalosporins on resistance in *Enterobacteriae* in food-producing animals.

In an experimental study, administration of a single dose of ceftiofur to turkey poults without detectable ceftiofur resistant strains did not result in the emergence of such strains (Poppe *et al*., 2005). The poults were dosed both with susceptible *S.* Newport and with *E. coli* carrying a large plasmid encoding for 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.

Tragesser et al (2006) studied the occurrence of ceftriaxone-resistant *E. coli* in dairy herds in Ohio, and linked the results to reported use of ceftiofur. 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). The mean within-herd prevalence was 40% for herds reporting ceftiofur use, 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 3rd 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).

Lowrance et al studied the influence of administration of ceftiofur crystalline free acid ('long acting') to steers (Lowrance *et al*., 2007). 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 streers served as controls. Administration of ceftiofur was associated with an increase in the proportion of *E. coli* resistant to ceftiofur during treatment in all treated groups. Almost all resistant isolates were co-resistant to at least chloramphenicol, streptomycin, sulphonamides and tetracycline, 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.

Persistence of ESBL 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). During the study period, all use of beta-lactam antimicrobials, apart from intramammary use of cefquinome, was stopped in an attempt to remove the selective pressure. However, 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 carrying a gene encoding CTX-M and in addition, streptomycin resistance.

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 flora (Hornish & Kotarski, 2002). However, the studies quoted above show that the concentrations are sufficient to select for *E. coli* with resistance to 3rd 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 noted repeatedly 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 commonly 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 frequent 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, *Salmonellae* and *E. coli* producing ESBL have emerged and increased in prevalence in poultry without prior use of cephalosporins (MARAN, 2005). It is probable that use of either beta-lactams such as amoxicillin, or non-beta-lactam antimicrobials, are contributing 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 isolated 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 *Salmonellae* 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 from Canada (Pitout *et al*., 2003).

Direct spread of CMY-2 producing multiresistant *Salmonella* Newport from cattle to humans has been documented (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 (Odds ratio 9) or matched healthy controls (Odds ratio 12) (Gupta *et al*., 2003).

Taken together, it is clear humans are exposed to cephalosporin resistant *Salmonellae* via food or via direct contact with infected animals, and that this may result in clinical infections.

Exposure to resistance genes from bacteria associated with animals

It has been suggested that in the normal human population, most resistant Enterobacteria in faeces come from contaminated food (Corpet, 1988). During the passage though the intestine, these 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 Amp-C 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 above, these genes are mostly carried on mobile genetic elements. The number of studies is still limited, as is the 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). Further, there are some reports indicating acquisition of resistance plasmids by *E. coli* and *Salmonella* sp. in the human gut (Su *et al*., 2003; Yan *et al*., 2005). A plasmid encoding an ESBL was identified in *E. coli* and *S.* Anatum, both isolated 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* also isolated 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. 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. However, any further expansion of the occurrence of ESBL or AmpC type 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. However, in very young patients and when fluoroquinolone resistance is present, 3rd generation cephalosporins are the drugs of choice.

In a study on outcomes 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 influenced by the fact that empiric treatment was 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. Emergence of multiresistant *Salmonellae*, with resistance also to cephalosporins and fluoroquinolones seriously limits the therapeutic options available.

The recent and rapid emergence of resistance of CTX-M type in *Enterobacteriaceae* isolated from human infections in Europe is a major public health concern (Canton & Coque, 2006; Livermore *et al*., 2007). The frequent occurrence of community-acquired infections and the frequent occurrence in *E. coli* is a particular worry. 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 a reservoir may exist in the community (Livermore *et al*., 2007). Considering the emergence of CTX-M producing *Salmonellae* and *E. coli* in animals as discussed above, it has been suggested that animals may be a reservoir and food a potential vector (Livermore *et al*., 2007).

The therapeutic options for infections with bacteria resistant to 3rd 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 mediated fluoroquinolone resistance, there is a high likelihood that this is not an effective alternative.

Animal health

For almost all of the indications for which ceftiofur or cefquinome are authorised for systemic therapy of food producing animals, equal or better alternatives are available. In particular, this is true for streptococcal infections, where cephalosporins have no advantage above benzylpenicillin in terms of efficacy or safety. In cattle, the only indication in which 3rd or 4th generation cephalosporins could be the sole alternative is severe clinical mastitis with 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 3rd and 4th generation cephalosporins (Rantala et al., 2002; Erskine *et al*., 2002; Shpigel *et al*., 1997). In horses, the only indication where cephalosporins can be regarded as critically important is neonatal sepsis in foals. In the treatment of this condition caused by *E. coli*, 3rd or 4th generation cephalosporins are alternatives for benzylpenicillin-aminoglycoside or trimethoprim-sulphonamide combinations. In many countries, resistance to both gentamicin and trimethoprim-sulphonamides in *E. coli* exist and in such cases, there will be few or no alternatives specifically authorised for use in horses available. Problems with invasive multiresistant *E. coli* in other food-producing species are not so critical, as either florfenicol or fluoroquinolones normally still remain active.

In conclusion, in most cases the direct impact of infections resistant to cephalosporins on animal health is low. However, emergence of resistance mediated by genes encoding ESBLs or AmpC is frequently linked to resistance to multiple other antimicrobials. 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 AND CONCLUDING REMARKS

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

In particular, genes coding for CTX-M type enzymes have rapidly emerged and spread not only in hospitals but also in the community. Production of ESBL in *Enterobacteriaceae* is often associated with resistance to other antimicrobials. The changing epidemiological pattern may be explained by many interacting factors. Most 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. The potential role of community reservoirs that might be 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 3rd 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 very rapid increases. A wide array of genes encoding ESBLs has emerged and is now present in enteric bacteria from animals. 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 currently undertaken by EFSA. Better data will be available in the future if all monitoring of resistance to 3rd generation cephalosporins is conducted, where applicable, in accordance with the standards developed by EFSA. Furthermore, an expansion of the monitoring to include also commensal *E. coli* from animals and food would provide data that is valuable for the assessment the reservoir of resistance genes.

The genes encoding resistance to 3rd and 4th 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 carried on plasmids and/or other transferable genetic elements, and are often linked to multiple other resistance genes. Spread of resistance can occur both though dissemination of clones, and though horizontal spread of, e.g. epidemic plasmids.

Data on the extent of use of 3rd and 4th 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 ESBL, and on potential differences between different doses and dosing regimens in this respect. In the future 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 3rd and 4th cephalosporins selects for resistance.

Use of 3rd and 4th 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. However, 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 3rd and 4th cephalosporins.

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. Therefore, it is likely that co-selection by antimicrobials other than cephalosporins have an important role. In particular, mass medication with various antimicrobials in animal husbandry may contribute to the occurrence and dissemination of resistance in exposed populations.

In addition, the importance of use of non-cephalosporins 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 though the environment.

Humans are exposed to cephalosporin-resistant *Salmonellae* via food or via direct contact with infected animals, and this can result in clinical infections. Further, humans may be exposed to animal bacteria with resistance genes coding for ESBL or AmpC type enzymes via direct contact, via contaminated food or indirectly though 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 contamination of food.

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

In the case of infections in humans with *Salmonellae*, *E. coli* or other *Enterobacteriaceae* that are resistant to 3rd and/or 4th generation cephalosporins, treatment alternatives are e.g. carbapenems, fluoroquinolones or aminoglycosides. However, 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 3rd and 4th generation cephalosporins is rapidly increasing in humans. Available evidence indicates that resistance to 3rd and 4th 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.

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RECOMMENDATIONS FOR ACTION

Cephalosporins are listed as critically important antimicrobials for human and veterinary use. The CVMP has taken note of SAGAM's review on cephalosporins and discussed the need for measures to be taken with regard to veterinary use of such products. Although it could be assumed that increased resistance levels recorded in human medicine are mainly due to comprehensive human use, 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 antimicrobial groups of specific concern due to its importance both in human and veterinary medicine.

The following recommendations are made:

- Systemic broad spectrum cephalosporins should be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow spectrum antimicrobials.
- The need of prophylactic use should always be preserved for specific circumstances and carefully considered in the conditions for authorisation and reflected in the SPCs.
- Use of systemic cephalosporins for groups or flocks of animals such as use of oral cephalosporins in feed or drinking water should be discouraged.
- 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 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 guideline SPC for antimicrobial products (EMEA/CVMP/SAGAM/383441/2005). This 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 isolated but a global approach to the problem of antimicrobial resistance is needed. Therefore, 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.

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Suggested action	Responsible body
• 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.	Farmer's organisations, competent authorities and related stakeholders.
• Veterinarians should be continuously educated on strategies to minimise antimicrobial resistance	Universities, Veterinary Associations, national authorities (e.g. granting veterinary authorisation)
• Emergence of cephalosporin resistance in pathogenic and indicator bacteria should be monitored and the need for interventions should be continuously evaluated.	The European Commission, EFSA, ECDC, Community Reference Laboratory, National Reference Laboratories and routine laboratories
• 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.	Member State Competent authorities
• All Member States should implement and enforce internationally recognised code of practice of rational and prudent use of antimicrobials (Codex code of practice to minimize and contain antimicrobial resistance CAC/RCP 61-2005; the OIE terrestrial code – chapter on antimicrobial resistance)	Member States
• Effect of chosen strategies should be monitored where possible in order to follow the efficacy of the measures.	Member States