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Reflection paper on the use of aminopenicillins and their beta-lactamase inhibitor combinations in animals in the European Union: development of resistance and impact on human and animal health

Draft

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Table of contents

Executive summary

The objective of this document is to review available information on the use of aminopenicillins and their beta-lactamase inhibitor combinations in veterinary medicine in the EU, their effect on the emergence of antimicrobial resistance (AMR) and the potential impact of resistance on human and animal health. The document provides information for the risk profiling, as recommended by the Antimicrobial Advice ad hoc Expert Group (AMEG) of the EMA, to assist with placing these substances within the AMEG's categorisation (EMA/AMEG, 2014). The focus of this paper is on veterinary aminopenicillins authorised in the EU, which are ampicillin (ATC J01CA01), amoxicillin (ATC J01CA04), and their beta-lactamase inhibitor combination amoxicillin-clavulanic acid (J01CR02).

The WHO classifies penicillins (natural, aminopenicillins and antipseudomonal) as critically important antimicrobials (CIA) for humans. According to the WHO, the CIA status is justified due to limited therapy options for listeriosis and infections caused by *Enterococcus* spp., and the likelihood of transmission of resistant *Enterococcus* spp. and Enterobacteriaceae, including both *Salmonella* spp. and *Escherichia coli,* from non-human sources to humans.

Although aminopenicillins are seldom among the sole treatment options, with the exception of for *Listeria* and enterococci, they are often used as first line antimicrobials for many infections in animals and humans. In animals aminopenicillins are used for infections caused by species belonging to Pasteurellaceae, *Streptococcus* spp., *Staphylococcus* spp., *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, *Clostridium* spp. and other anaerobic species, *Bordetella bronchiseptica* and species belonging to the Enterobacteriaceae. Aminopenicillins and their inhibitor combinations are very valuable drugs for treating respiratory infections in humans caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Branhamella catarrhalis*. Due to the abundant presence of beta-lactamases in *E. coli* and in many other Enterobacteriaceae, aminopenicillins are combined with beta-lactamase inhibitors for the treatment of infections caused by these bacteria. Inhibitor combinations can also be useful in certain infections caused by ESBL-producing *E.coli* provided that an isolate is susceptible to the combination *in vitro*. The combination is ineffective against AmpC-mediated resistance.

Ampicillin, amoxicillin, and to a lesser extent amoxicillin-clavulanic acid combinations have been widely used for decades for the treatment of infections in several animal species in European countries. Measured in mg/PCU (population correction unit), penicillins were the second most used antimicrobial class in food-producing animals in the EU in 2015 and accounted for 25% of the total sales. Aminopenicillins (amoxicillin) made up the major proportion (88%) of the total penicillin use, while their inhibitor combinations formed a very limited fraction of the total penicillin use. There are substantial differences between the uses of different beta-lactam drug classes in animals in Nordic countries, where benzyl penicillin and its pro-drugs dominates, vs. in other European countries, where aminopenicillins are the prevailing beta-lactams used. This may be due to differences in treatment guidelines, availability of authorised products, production systems (including dominant animal species), herd sizes, disease occurrences, and production facilities, or even manners and habits of antimicrobial usage (e.g. whether mass medication is favoured instead of individual treatment).

According to the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) summary report on 2016 data, the most commonly used antimicrobials in human medicine were penicillins (ATC J01C), however data that specify the human use of those aminopenicillins (J01CA01, J01CA04) and inhibitor combinations (J01CR02) that also have authorisation for animals, are not readily available. If

human and animal beta-lactam use are compared as mg/kg of estimated biomass, human use is approximately twice that for animals (80 vs. 40 mg/kg of estimated biomass).

Aminopenicillin (or penicillin) resistance has not yet been described in group A, B, C or G beta-hemolytic streptococci, regardless of origin (animal/human). Aminopenicillin resistance in clinical *Listeria monocytogenes* is very rare. Regarding other streptococci, enterococci (mainly *E. feacalis*) and Pasteurellaceae, penicillin/aminopenicillin non-susceptibility levels are generally low but vary by country, production system, and animal and bacterial species. More than 75% of human *E. faecium* isolates show resistance to ampicillin while less resistance has been detected in isolates of animal origin.

Aminopenicillins are able to select not only for aminopenicillin resistance, but also co-select for other resistances, including to extended spectrum cephalosporins. It is clear that resistant organisms, such as MRSA and those producing ESBL/AmpC, are transferred between animals and humans but both the direction and magnitude of transfer are often difficult to prove or quantify. The pathway from animals to humans is obvious for zoonotic organisms, such as salmonellae and campylobacters, which cause illness in humans. Also the origin of certain LA-MRSA clones is proven to be in livestock, but for commensals that are part of the normal microbiota, the role of animals as the source of resistance is unclear. Although identical clones, the same resistance genes and mobile genetic elements have been detected in many bacteria of animal and human origin, the effect of veterinary antimicrobial use on their presence or emergence in the human population is equivocal. For example, studies utilising new sequencing methods have revealed high genetic diversity between the isolates from different sources indicating that veterinary antimicrobial use might not have a major impact on selection of ESBL/AmpCs detected in humans. Resistance to aminopenicillins is common in *E. coli* of animal and human origin, but resistance levels to the inhibitor combinations in bacteria of animal origin are lower.

Considering that aminopenicillin resistance is at a very high level in some organisms and that aminopenicillins have been extensively used for decades both in animals and humans, it is currently impossible to estimate to what extent the use of these substances in animals, could create negative health consequences to humans at the population level. There are studies that have attempted to address these challenges. In general, risk estimates range from a few additional infections per million at risk to thousands, depending on antimicrobial substance and pathogen in question. Individual risk estimates following assessments of aminopenicillin resistance exposure via the food might be low, especially if good food hygiene practices are followed. However other routes of exposure should be taken into consideration (such as direct contact).

Although the direct AMR risk to humans from the veterinary use of aminopenicillins would be lower compared to the risk from their use in human medicine, it is evident that veterinary aminopenicillin use increases the selection pressure towards AMR and jeopardizes at least animal health and welfare. Based on an assessment of current use and resistance profiling, it may be possible to make recommendations to limit the further development of resistance to both aminopenicillins and related classes of antimicrobials and to maintain the efficacy of these valuable drugs in the future. Tools include improvements in hygiene in animal husbandry, use of vaccinations, proper diagnostics and avoidance of use of antimicrobials prophylactically to animals having no signs of infection. Also, the route of administration should be considered to reduce the selection pressure in the gut microbiota. For example group medication of food-producing animal flocks by the oral route facilitates the selection and spread of resistance and attempts to reduce such use are needed.

CVMP Recommendations for action

Proposal on categorisation for consideration by AMEG

- The AMEG categorisation considers the risk to public health from AMR due to the use of antimicrobials in veterinary medicine. The categorisation is based primarily on the need for the antimicrobial in human medicine, and the risk for spread of resistance from animals to humans. Aminopenicillins are important in human medicine in terms of their high extent of use to treat a variety of important infections, although there are alternatives of last resort. Aminopenicillins have potential to select LA-MRSA and resistance in foodborne zoonotic pathogens, including *Salmonella* spp., which can be transferred to humans from livestock. In addition, resistance to aminopenicillins is very frequent in commensal Enterobacteriaceae from food-producing animals in the EU, which could act as a reservoir for resistance genes that may be transferred to pathogenic bacteria in humans. However, the high extent of aminopenicillin use in humans itself provides a selection pressure for resistance in the human microbiota and the significance to public health of additional aminopenicillin resistance transferred from animals is considered to be low. Although amoxicillin beta-lactamase inhibitor combinations have very low use in food-producing animals, AmpC/ESBL resistance mechanisms, which also confer resistance to 3rd- and 4th-generation cephalosporins, have emerged in Enterobacteriaceae from animals in recent years and the combination has the potential to select further these types of resistance than amniopenicillins alone.

- It should also be considered that aminopenicillins have been widely used for decades in veterinary medicine in the EU, and that they are categorised as veterinary CIAs by the OIE on the grounds that they are very important in the treatment of many diseases in a broad range of animal species.

- All these factors should be taken into account for the AMEG's categorisation, which is currently under review. It is suggested that the AMEG could give consideration to a further stratification of the categorisation to allow a distinction in the ranking between those substances currently in Category 2 (fluoroquinolones, 3rd- and 4th-generation cephalosporins and colistin, for which there are fewer alternatives) and the amoxicillin-clavulanate combinations, and between the latter and the straight aminopenicillins. Amoxicillin-clavulanate has wider spectrum and thus it is likely that it has higher chance to select multidrug resistant organisms compared to aminopenicillin alone. In case accumulating evidence from future scientific research indicates that veterinary use of aminopenicillins poses an added threat to public health due to animal-to-human resistance transfer, it could then be considered if a distinction in the categorisation should be made between straight aminopenicillins and narrow-spectrum penicillins.

Considerations for Marketing Authorisations and summary of product characteristics (SPCs)

- Current indications should be reviewed in relation to authorised dosing regimens in order to ensure achievement of sufficient pharmacokinetic/pharmacodynamic (PK/PD) targets and subsequently to minimise the risk for resistance selection, especially concerning inherently less susceptible organisms such as Enterobacteriaceae and *Bordetella bronchiseptica.*

- Since there is great variation in dosing regimens between similar products authorised in the EU, these should be reviewed to harmonise schemes and ensure effective dosing.

- In reference to the above recommendations and the scope of any referral procedures for aminopenicillins and their combinations, review of groups of products would be prioritised according to their relative risk to animal and public health.
- Based on high levels of resistance in Enterobacteriaceae, it is recommended that the use of aminopenicillins for the treatment of infections caused by such pathogens should be based on susceptibility testing.

Responsible parties: CVMP, Regulatory Agencies, Marketing Authorisation Holders (MAHs)

Need for research

- Susceptibility testing should be standardised and veterinary clinical breakpoints should be established for aminopenicillins to enable proper interpretation of susceptibility tests.
- There is need for a harmonised European wide surveillance scheme to encompass target pathogens from food-producing and companion animals.
- The same resistance genes carried by the same mobile genetic elements have been found in isolates from animals and humans and there is potential for transmission of resistance from animals to humans. Further research is needed to elaborate on the link between the use of antimicrobials in animals and the impact on public health.

Responsible parties: European Commission, EURL-AMR, EFSA, VetCAST

1. Background

As part of the EC Action plan against antimicrobial resistance (AMR), the European Commission (EC) requested advice from the European Medicines Agency (EMA) on the impact of the use of antimicrobials in animals on public and animal health and measures to manage the possible risks it may cause to humans. This is because aminopenicillins, especially those combined with beta-lactamase inhibitors, have a spectrum of activity which overlaps with 2nd- and to lesser extent 3rd-generation cephalosporins. Thus they might have the ability to select and facilitate the spread of bacteria carrying extended spectrum beta-lactamases (ESBLs), similarly to 3rd- and 4th-generation cephalosporins and fluoroquinolones (EMA/AMEG, 2014). WHO classifies penicillins (natural, aminopenicillins and antipseudomonal) as critically important antimicrobials (CIA) for human medicine (WHO, 2017).

As in the concept paper published by the CVMP (EMA/CVMP, 2015a), the focus of this paper is on veterinary authorised extended-spectrum penicillins in the EU, which are the aminopenicillins ampicillin (ATC J01CA01) and amoxicillin (ATC J01CA04), and the beta-lactamase inhibitor combination amoxicillin-clavulanic acid (J01CR02). The objective of this document is to review available information on the use of these substances in veterinary and in human medicine in the EU, the influence that veterinary use in particular has on the emergence of AMR and its potential impact on human and animal health. The document provides information for risk profiling, as recommended by the Antimicrobial Advice ad hoc Expert Group (AMEG), which will allow these substances to be placed within the AMEG's categorisation. The AMEG is currently reviewing the criteria for its categorization and could give consideration to its further stratification.

2. General drug characteristics

2.1. Structure and mechanism of action

Ampicillin, amino-p-hydroxy-benzyl penicillin, was the first semisynthetic penicillin introduced into clinical use in 1961 by Beecham Laboratories. It was followed by amoxicillin in the early 1970's (Rolinson, 1998). Amoxicillin has an otherwise identical structure to ampicillin, except for an additional hydroxyl group attached to a phenyl ring of the side chain. Discovery of the active moiety, 6-aminopenicillanic acid nucleus (6-APA), from the penicillin molecule enabled the development of semisynthetic penicillins with enhanced spectrum of activity for Gram-negative bacteria. In 6-APA, a beta-lactam ring is attached to a thiazole ring. The structure of the side chain linked to the amino group of the 6-APA determines the pharmacokinetic properties and antimicrobial activity of the drug (Rolinson, 1998).

Aminopenicillins inhibit the activity of the transpeptidase and other peptidoglycan-active enzymes that catalyse the cross-linking of the glycopeptide units in the bacterial cell wall. Target enzymes are called penicillin binding proteins (PBPs). Aminopenicillins bind to PBPs by mimicking the structure of the natural substrate (D-alanyl-D-alanine) of the enzymes. This leads to incomplete cross-linking of peptidoglycan building blocks and induces osmotic lysis of the bacterial cell due to loss of rigidity of the peptidoglycan layer. The action is bactericidal, but affects only actively dividing bacterial cells (Giguère et al., 2013). The composition of PBPs in the bacterial species in question partly explains the spectrum of different beta-lactams, for example, enterococci are naturally susceptible to aminopenicillins but not to cephalosporins (Kristich and Little, 2012).

Due to the emergence of beta-lactamase mediated resistance that impaired the efficacy of aminopenicillins, the search for beta-lactamase inhibitors started in the late 1960s (Rolinson, 1998). Clavulanic acid is a beta-lactamase inhibitor with a beta-lactam-like structure. It is produced by *Streptomyces clavuligerous* (Brown et al., 1976). Clavulanic acid and other beta-lactamase inhibitors with a beta-lactam core, such as sulbactam and tazobactam, have only a weak antimicrobial activity of their own. In combination products, a beta-lactamase inhibitor binds irreversibly to bacterial beta-lactamases blocking their activity, while the actual beta-lactam component maintains its activity against bacteria. Amoxicillin-clavulanic acid was the first beta-lactam - beta-lactamase inhibitor combination coming into the market in 1981 (Bush, 1988). Clavulanic acid binds covalently to several bacterial beta-lactamases including type II, III, IV and V beta-lactamases, as well as staphylococcal penicillinases, but it is ineffective against class I cephalosporinases (AmpC type) and carbapenemases (Drawz and Bonomo, 2010). In veterinary therapeutic products amoxicillin is combined with clavulanic acid usually in a 4:1 ratio. There are no other beta-lactam beta-lactamase inhibitor combinations authorized in veterinary medicine in the EU.

2.2. Antimicrobial spectrum

The antimicrobial spectrum of ampicillin and amoxicillin against Gram-positive bacteria covers, among others, the following Gram-positive genera: *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Listeria*, *Actinomyces*, *Trueperella*, *Corynebacterium*, and *Erysipelothrix*. Compared to natural penicillins, aminopenicillins are more hydrophilic and thus are able to diffuse better through the outer membrane of the Gram-negative bacteria. Of Gram-negative genera, *Haemophilus*, *Histophilus*, *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Neisseria*, *Moraxella*, *Borrelia*, and *Leptospira* are usually susceptible. Of the Enterobacteriaceae, *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* species are susceptible

unless they have acquired resistance mechanisms. Susceptible anaerobes include, among others, anaerobic Gram-positive cocci, *Clostridium* spp., *Fusobacterium* spp., *Prevotella* spp. and *Porphyromonas* spp.

Ampicillin and amoxicillin are ineffective against *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., indole-positive *Proteus* spp., *Acinetobacter* spp. and *Pseudomonas* spp. due to intrinsic resistance mechanisms in these species. Also *Bordetella* ssp., rickettsia, mycoplasma and mycobacteria are resistant (Giguère et al., 2013).

Staphylococcal penicillinases and beta-lactamases produced by Gram-negative bacteria inactivate ampicillin and amoxicillin. Thus aminopenicillins are often combined with a beta-lactam inhibitor or replaced by cephalosporin group antimicrobials. In the EU, the only veterinary authorized inhibitor combination is amoxicillin clavulanic-acid. It has a spectrum of activity corresponding to that of 2nd-generation cephalosporins and covers also *Klebsiella* spp., *Bordetella* spp., *Bacteroides* spp. and indole positive *Proteus* spp. (Giguère et al., 2013).

There is great variation in relative susceptibility to aminopenicillins between bacterial genera. The wild type *Streptococcus* spp., *Actinomyces spp.*, *Clostridium perfringens*, *Listeria* spp., *Haemophilus* spp., *Histophilus* spp., *Moraxella* spp., and *Pasteurella* spp, have the lowest minimal inhibitory concentrations (MICs), ≤ 1 mg/L. MICs for the wild type *Enterococcus* spp. range from 0.25 to 4 mg/L, while the wild type *E. coli* isolates have relatively high MICs, 1 - 8 mg/L, both for ampicillin and amoxicillin clavulanic acid. The same applies to *Salmonella* Enteritidis, while other salmonellae are slightly more susceptible (https://mic.eucast.org/Eucast2/). *Klebsiella* species are intrinsically resistant to ampicillin or amoxicillin (MICs ≥ 4 mg/L), but when amoxicillin is combined with clavulanic acid, the MICs of the main population range from 1 - 8 mg/L (www.eucast.org).

2.3. Pharmacodynamics

Ampicillin and amoxicillin are bactericidal and their effect is time-dependent. Optimal killing occurs if bacteria are exposed to an antimicrobial concentration exceeding 1 - 4 x the MIC for sufficient time between the dosing intervals. Thus, for time dependent drugs, a time above the MIC (T>MIC) is the best pharmacokinetic/pharmacodynamic (PK/PD) parameter predicting microbiological and clinical efficacy. For beta-lactams, the target T>MIC is 50 – 80% of the dosing interval (Toutain et al., 2002). Beta-lactams possess significant post-antibiotic effect (PAE) against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*, although the length of the PAE ranges widely, between 0.5 - 6 hrs (Preston and Drusano, 1999). Gram-negative bacteria show no considerable PAE effect after exposure to ampicillin or amoxicillin (Brown et al., 1976). Therefore, for infections caused by Gram-negative bacteria, a shorter dosing interval is recommended compared to infections caused by Gram-positive bacteria (Toutain et al., 2002). Aminopenicillins penetrate poorly into phagocytes and hence have limited ability to kill intracellular pathogens like *Salmonella* spp. (Mandel and Petri Jr, 1996).

Although the antimicrobial spectrums of ampicillin and amoxicillin are nearly identical, an early study proved that at concentrations close to the MIC, ampicillin shows a slower killing rate *in-vitro* than amoxicillin against *E. coli* and *Salmonella* Typhi due to slower lysis of bacterial cells (Basker et al., 1979). The same has been observed *in vivo* in mice an experimental intra-peritoneal infection model in which amoxicillin was observed to be more effective than ampicillin in protecting the mice from the lethal effects of the *E. coli* infection - regardless that concentrations of both compounds in the body fluids were equal (Comber et al., 1977). Amoxicillin induced the formation of rapidly lysing spheroplast

forms of the bacterial cell while ampicillin resulted in slowly lysing long bacterial filaments (Comber et al., 1977).

Paradoxically, increasing the concentration of beta-lactam antimicrobials above the optimal killing concentration can lead to impaired killing of bacteria. This is known as the Eagle effect, and is sometimes observed *in vitro* with beta-lactams against Gram-positive cocci and rods (Grandière-Pérez et al., 2005; SHAH, 1982). The effect is probably due to binding of a beta-lactam to other than primary target PBPs, so preventing bacterial cell wall synthesis and multiplication, while beta-lactams are active only against actively dividing cells. The clinical impact of this phenomenon is unclear (Lamb et al., 2015).

2.4. Pharmacokinetics

Although amoxicillin and ampicillin are closely related in their structure as well as in chemical and physical properties, the extent of absorption after oral dosing differs markedly between these molecules. Generally speaking, the amoxicillin serum drug concentration is twice that of ampicillin with the same dose. The speed of bactericidal action of amoxicillin is more rapid and complete compared to ampicillin when administered at the same dose (Prescott, 2013). In monogastric animals 33 – 92% of the dose is absorbed after oral administration of amoxicillin. The comparative figure for ampicillin is 30-55%. The absorption of amoxicillin is unaffected by feeding in pigs (Agersø and Friis, 1998a), dogs and humans, unlike ampicillin (Watson and Egerton, 1977). Aminopenicillins cannot be administered orally for adult ruminants, horses or animal species [such as rabbits] that are prone to severe disturbance of their gut microbiota. The volume of distribution is 0.2 - 0.3 L/kg depending on species. The drug is distributed widely in the extracellular fluids of many tissues including lungs, muscle, bile, peritoneal and pleural fluid, and synovial fluid. If the meninges are inflamed, therapeutic drug concentrations may be achieved in the cerebrospinal fluid. In milk the concentration is low, approximately one fifth of that in serum. Protein binding varies between 8 – 20% depending on animal species. The elimination half-life is 45 - 90 min, being longest in cattle, although it can be prolonged by the use of sustained release drug formulations. Elimination occurs through renal excretion mainly as active drug (Prescott, 2013). The pharmacokinetics of clavulanic acid resembles that of amoxicillin. Clavulanic acid is readily absorbed after oral administration. It is widely distributed into extracellular fluids, but poorly into milk or inflamed cerebrospinal fluid. Its half-life is approximately 1.25 hrs and it is excreted primarily in urine as unchanged drug (Prescott, 2013). Achievable drug concentrations after various formulations and dosages in different animal species are summarised below and are presented as mg/L (instead of µg/ml) in order to facilitate comparison of bacterial susceptibilities in relation to achievable drug concentrations *in-vivo*.

Pigs: An intra-muscular (i.m.) dose of 10 mg/kg ampicillin sodium resulted C_{max} of 12 mg/L and 14 mg/L in plasma of healthy and *Streptococcus suum* [*Streptococcus suis*] infected 2-month-old pigs, respectively. The half-life was shorter in the latter group (0.76 vs. 0.57 h) (Yuan et al., 1997). In three-week old piglets a peak plasma concentration of 7 mg/L was observed after i.m. injection of 17.6 mg ampicillin trihydrate /kg (Apley et al., 2007). With the oral dose of 20mg/kg ampicillin, a high drug concentration (720 mg/L) in caecal fluid was achieved, while twice that dose intramuscularly resulted in a concentration of only 15 mg/ml (Escoula et al., 1982). A conventional amoxicillin-trihydrate formulation, dosed at 14.7 mg/kg i.m. produced a peak concentration of 5.1 mg/L but with a sustainable release (LA) formulation (dose 14.1 mg/kg), the peak concentration was only 1.7 mg/L. Oral administration of amoxicillin produced very low peak plasma concentrations in pigs, ranging from 0.2 to 3.1 mg/L depending on dose (10-23 mg/kg), and on whether the drug was given as oral bolus,

or in feed or drinking water (Agersø and Friis, 1998a; Agersø et al., 1998; Godoy et al., 2011). An oral amoxicillin clavulanic-acid bolus of 25 mg/kg (5 mg/kg clavulanic acid) produced an amoxicillin peak concentration of 3.1 mg/L and clavulanic-acid concentration of 2.4 mg/L (Reyns et al., 2007). In healthy pigs, the tissue to plasma ratios (based on AUC values) of amoxicillin were 0.33 for bronchial secretions, 0.37 for bronchial mucosa, 0.39 for lung tissue, and 0.68 for lymph nodes (Agersø and Friis, 1998b).

Cattle and other ruminants: In cows and calves, a dose range of 10 - 11 mg/kg of ampicillin trihydrate i.m. resulted in C_{max} 1.6 – 2.2 mg/L in plasma within couple of hours (Credille et al., 2015). In dairy cows the peak concentration in milk was at a similar level as in plasma, but several times higher drug concentrations (55 - 75 mg/L) were detected in lochia (Credille et al., 2015). In calves, peak concentrations in synovial fluid ranged from 2.7 (healthy) to 3.5 mg/L (suppurative), with peaks in synovial fluid following later compared to those in plasma (Brown et al., 1991). With a dose of 15 mg/kg of long acting amoxicillin trihydrate formulation in ruminant calves, C_{max} of 2.9 mg/kg in plasma was achieved within 1.3 hours, while in exudate and transudate fluids the respective values were 1.29 and 1.45 mg/L within 10.6 and 14.5 hours, respectively (Lees et al., 2015). Based on conservative PK/PD modelling and Monte Carlo simulation, the doses predicted to lead to bacterial eradication over the 48-hour period in ruminant calves (90% probability for the plasma drug concentration to exceed the PD endpoint for efficacy) ranged from 37.5 (*Pasteurella multocida*) to 43.6 mg/kg (*Mannheimia haemolytica*); far higher than the authorised doses for this indication (Lees et al., 2015). Oral administration of amoxicillin trihydrate to pre-ruminant calves at the dose of 10 mg/kg produced C_{max} 2.08 mg/L in plasma within 2.5 hours, while a 10 - 20 mg/kg dose of amoxicillin clavulanic-acid resulted in an amoxicillin C_{max} of 1.98 - 3.26 mg/L (Soback et al., 1987).

In sheep, a dose of 15 mg/kg by intra-muscular administration of conventional amoxicillin trihydrate product resulted in a C_{max} 5.3 mg/L in plasma, but with a sustainable release formula, the respective value was 2.7 mg/L (Delis et al., 2009). Another study reported that 10 mg/kg of amoxicillin-trihydrate i.m. to sheep produced a C_{max} of only 2.48 mg/L in plasma, but with the same dose of amoxicillin sodium the Cmax was 13.42 mg/L (Fernandez et al., 2007). According to a PK/PD simulation, at the dose of 15 mg/kg of amoxicillin trihydrate once daily, a T>MIC of 69%-75% in sheep serum or tissue cage fluid was achieved, provided that the MIC of the pathogen was ≤ 1 mg/L (Delis et al., 2010). A pharmacokinetic study with intravenous amoxicillin-clavulanic acid revealed that in sheep the elimination half-lives of amoxicillin and clavulanic acid were slightly longer (1.43 and 1.16 hours, respectively) than in goats (1.13 and 0.85 hours, respectively), but volumes of distribution were similar (Carceles et al., 1995).

Poultry: In broiler chickens at the dose of 10 mg/kg of amoxicillin, the elimination half-life ranged from 1.07 to 1.13 hours depending on the route of administration. Bioavailability was 77% and 61% after intra-muscular and oral dosing, respectively. Due to high clearance, the plasma drug level was maintained above 0.25 mg/L for only 6 hours after both routes. Only 8.3% of amoxicillin was observed to bind to plasma proteins in this species (El-Sooud et al., 2004). Enteric coccidiosis may result in lower peak amoxicillin concentrations in infected compared to healthy chickens (Kandeel, 2015). In turkeys, an oral dose of 12.5 mg/kg of amoxicillin clavulanic-acid (10 mg amoxicillin, 2.5 mg clavulanic acid) resulted in C_{max} of 3.2 mg/L and 1.05 mg/L of amoxicillin and clavulanic-acid in plasma, respectively (Jerzsele et al., 2011).

Horses: In adult horses, ampicillin trihydrate at 20 mg/kg i.m. resulted in a peak serum concentration of 2.49 mg/L within six hours. In synovia and peritoneal fluid the peak concentrations were 1.65 mg/L and 1.81 mg/L within 6 and 4 hours, respectively. Urine concentration of ampicillin was relatively high,

with a C_{max} of 1200 mg/L at 4 hours. Ampicillin was still detectable at 48 hours in body fluids (Brown et al., 1982). The pulmonary epithelial lining fluid (PELF) to plasma ratio was 0.4 after 15 mg/kg of intra-venous ampicillin sodium injection while the observed C_{max} in PELF with this dosage was 3.96 mg/L. After 12 hours, a concentration of 0.32 mg/L of ampicillin was still observed in PELF (Winther et al., 2012). In 3 - 30 day-old foals, 22 mg/kg of amoxicillin sodium i.m. injection produced a C_{max} of 17 - 23 mg/L in plasma; the lowest level was observed in 3-day-old foals (Carter et al., 1986). With oral administration of 20 - 30 mg/kg amoxicillin sodium syrup to neonatal foals, concentrations in plasma of 6.3 - 12.1 mg/L were achieved with 36 – 42% bioavailability, while at 6 hours the concentrations were 0.9 - 1.66 mg/L (Baggot et al., 1988). Due to very low oral bioavailability (0 – 5%) and a risk for severe disturbance of gut microbiota, oral administration of aminopenicillins is contraindicated to adult horses.

Dogs and cats: Amoxicillin trihydrate administered orally at 20 mg/kg to dogs produced a C_{max} of 18.1 - 20.7 mg/L within 1.4 - 2 hours, depending on formulation (tablets, oral bolus, drops) (Chicoine et al., 2007; Küng and Wanner, 1994; Watson et al., 1986) while an oral dose of 10 mg amoxicillin trihydrate/kg in tablet form resulted in a C_{max} 8.1 mg/Lin plasma (Watson et al., 1986). In cats, after an oral dose of 11 mg amoxicillin trihydrate/kg as tablets, C_{max} in plasma was 9.9 mg/L (Chicoine et al., 2007). After an oral dose of 25 mg/kg of amoxicillin clavulanic-acid to dogs the C_{max} for amoxicillin was 11 mg/L, while C_{max} for clavulanic-acid was 2.06 mg/L, with half-lives of 1.5 and 0.76 hours, respectively (Vree et al., 2003). In cats slightly higher C_{max} values were achieved with the same dose of this combination with half-lives of 1.2 and 0.6 hours for amoxicillin and clavulanic-acid, respectively (Vree et al., 2002). According to the SPC of one amoxicillin-clavulanic acid injectable formulation, an injection of 8.85 mg/kg of the product (of which 1.75 mg/kg is clavulanic acid) subcutaneously produces Cmax values 2.8 (amoxicillin) and 2.4 mg/L (clavulanic acid) in dogs, while respective C_{max} values in cats are 4 and 3 mg/L.

3. Resistance mechanisms and susceptibility testing

3.1. Resistance mechanisms

3.1.1. Enzymatic degradation of beta-lactams by beta-lactamases

The most important mechanisms of resistance to the beta-lactam antimicrobials are the beta-lactamase enzymes that catalyse hydrolysis of the beta-lactam ring. There is a very wide variety of different beta-lactamases with varying substrate specificity (Bush, 2013). Aminopenicillins are prone to hydrolysis by all clinically relevant beta-lactamases. Clavulanic acid inhibits many, but not all of these. The genes coding beta-lactamases are ancient and have been detected in many ecological niches, both urban and rural, and in several bacterial species, both Gram-positive and Gram-negative, worldwide (Davies and Davies, 2010).

To date, more than 1300 different types of beta-lactamases have been characterised (http://www.lahey.org/studies/). Traditionally they have been classified either according to functional features of the enzymes (Bush-Jacoby classification) or their amino-acid structure (Ambler classification). Functional classification of beta-lactamases was updated in 2010 by Bush and Jacoby (Bush and Jacoby, 2010) and is summarised below and in

Table **1**.

Group 1 cephalosporinases [so called AmpC enzymes] are usually not inhibited by clavulanic acid, sulbactam, or tazobactam. Examples of Group 1 enzyme families are CMY, ACT, DHA, FOX, and MIR. Apart from penicillins and aminopenicillins, they hydrolyse cephalosporins; especially cephamycins such as cefoxitin and cefotetan; oxyiminocephalosporins such as ceftazidime, cefotaxime, and ceftriaxone; and monobactams such as aztreonam (Jacoby, 2009). They have been recognised since 1989 and can be carried by plasmids (horizontally transferable to neighbouring bacteria of related species), although they can also be chromosomally (vertically transferable within the same clonal lineage) encoded by some species belonging to Enterobacteriaceae. An example of the chromosomal presence of the AmpC is a low level AmpC production detected in *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and a several other species (Bush and Jacoby, 2010). Low level chromosomal AmpC production can be induced to a high-level by aminopenicillins and clavulanic-acid. Also other beta-lactams can act as inducers for AmpC beta-lactamase. Hyper production of AmpC enzymes is due to mutations in genes regulating the enzyme expression (Jacoby, 2009). The original source for plasmid-encoded AmpC genes are in those bacterial species in which chromosomal AmpC-genes are common; for example: *Citrobacter freundii* (CMY), *Morganella morganii* (DHA), *Hafnia alvei* (ACC), *Aeromonas* spp. (CMY, FOX) and *Enterobacter* spp. (ACT/MIR) (Rossolini and Docquier, 2006).

Group 2 serine beta-lactamases represent the largest beta-lactamase group. A sub-group of enzymes belonging to this group are penicillinases with limited spectrum of hydrolytic activity, such as penicillinases of staphylococci and some other Gram-positive cocci. They hydrolyse only natural penicillins and aminopenicillins. Another sub-group belonging to Group 2 hydrolyse penicillins and early cephalosporins. Examples include plasmid-mediated TEM-1, TEM-2 and SHV-1 enzymes that were detected in the 1970s and early 1980s. The third sub-group includes classical ESBL-enzymes, for example ESBL-variants of TEM and SHV families, as well as CTX-M type enzymes. In addition the group contains serine carbapenemases, such as KPC and certain carbapenem destroying variants belonging to SHV family. The fourth sub-group in Group 2 are OXA-type beta-lactamases of which many variants are capable of hydrolysing carbapenems (Bush and Jacoby, 2010; Liakopoulos et al., 2016; Munoz-Price et al., 2013).

Group 2 serine beta-lactamases are usually carried by plasmids (Bush and Jacoby, 2010). The enzymes of this class are generally inhibited by clavulanic acid and other similar inhibitors, although there are SHV and KPC variants that are inhibitor resistant (Papp-Wallace et al., 2015; Winkler et al., 2015). The location of genes coding OXA-type beta-lactamases can be chromosomal or in plasmids. These enzymes have typically been found in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, but have also been described in some species belonging to *Enterobacteriaceae*. The substrates of OXA-type carbapenemases are diverse and generally include benzylpenicillin, aminopenicillins, piperacillin and ticarcillin, narrow-spectrum cephalosporins (such as cephalothin and ceafaloridine) in addition to carbapenems. They have low hydrolytic activities against imipenem and meropenem, but they do not affect extended-spectrum cephalosporins or aztreonam, or these are only poorly hydrolysed (Walther-Rasmussen and Høiby, 2006).

Group 3 includes metallo-beta-lactamases that are capable of hydrolysing carbapenems, extended spectrum cehpalosporins in addition to many other beta-lactams, including aminopenicillins. They are not inhibited by clavulanic acid, but are inhibited by metal-ion chelators, such as EDTA. These enzymes require zinc for function at the active site. They are spread in plasmids. Enzyme families belonging to Group 3 metallo-beta-lactamases include IMP, VIM and NDM (Bush and Jacoby, 2010).

3.1.1.1. Evolution of beta-lactamases in post-antibiotic era

Staphylococcal beta-lactamases appeared only within a few years after introduction of penicillin in the 1940s (Hall and Barlow, 2004). The strains with this mechanism quickly disseminated in hospitals and became very common compromising treatment outcome with penicillin. Emergence of penicillin resistant staphylococci led to discovery of new antimicrobial agents that were stable to staphylococcal penicillinases. Staphylococcal beta-lactamase is a narrow-spectrum spectrum enzyme capable of hydrolysing penicillin G and V, as well as aminopenicillins. Interestingly, it has remained stable despite heavy exposure of several penicillinase-resistant beta-lactams over the decades (Medeiros, 1997). This is in contrast to beta-lactamases of Gram-negative species in which there is a very wide variety of different beta-lactamases with varying substrate specificity (Bush, 2013). Within the last few decades numerous different types of beta-lactamases with ever more wide spectrum have emerged, seriously compromising the usefulness of beta-lactams (Al-Bayssari et al., 2015).

Enterobacteriaceae isolates from the pre-antibiotic era (1917-1954) carried conjugative plasmids of the same incompatibility groups as respective bacterial species of the modern era (Hall and Barlow, 2004). However, beta-lactamase mediated resistance was not yet present in these. Ampicillin came into markets in 1961, and soon after this 1st-generation cephalosporins were introduced. Plasmid borne TEM-1 and SHV-1 enzymes, that hydrolyse penicillins, aminopenicillins, and 1st- and 2nd-generation cephalosporins, were discovered soon after introduction of these drugs, in 1963 and 1974, respectively (Medeiros, 1997).

In the late 1970's and 1980's amoxicillin clavulanic-acid and other beta-lactam beta-lactamase inhibitor combinations as well as several extended spectrum 3rd-generation cephalosporins were approved into clinical use (Medeiros, 1997). Due to high frequency of resistant bacteria to older agents, the use of these drugs and monobactams increased rapidly in 1980's. As a result of changed selection pressure, and due to high plasticity of beta-lactamase enzymes, several new variants of TEM and SHV capable of hydrolysing extended spectrum cephalosporins were observed in 1980s, and a novel group of extended spectrum beta-lactamases, CTX-M, in 1987 (Hall and Barlow, 2004).

By the mid-1980's, it was noted that bacteria carrying chromosomal AmpC cephalosporinases (e.g. *Enterobacter* spp., *Citrobacter* spp.) developed clinical resistance to many newer beta-lactams during therapy with these drugs. The resistance was due to high expression of genes coding cephalosporinases. It was later observed that elevated expression of these enzymes can be due to reversible induction, i.e. elevated expression persists as long as the inducer is present. Many beta-lactams like benzylpenicillin, ampicillin, amoxicillin, and cephalosporins (e.g. cefazolin and cephalotin) are strong inducers of AmpC enzymes. Also clavulanic-acid, although having little inhibitory effect on AmpC enzymes on its own, can paradoxically increase these enzymes in an inducible bacteria (Jacoby, 2009). A second mechanism is continuous hyper-production of the enzyme due to stable derepression of beta-lactamase secretion by spontaneous mutations in genes regulating the gene expression. Beta-lactams vary in their ability to select these mutants (Sanders, 1987). Mutants emerged in hospital environments and were recognised as significant nosocomial pathogens (Sanders, 1987). After some years, in 1989, the first plasmid-mediated cephalosporinase was observed in *K. pneumoniae* (Jacoby, 2009). Since then, this type of resistance, either chromosomal or plasmid-borne, has been detected worldwide in several bacterial species, mainly in Enterobacteriaceae, of human and animal origin. Although resistance due to plasmid-mediated AmpC enzymes can be less common than extended-spectrum β-lactamase production it is harder to detect and broader in spectrum (Jacoby, 2009).

In general, in 1961 transferable beta-lactamase resistance was an unknown phenomenon while forty years later already 200 different beta-lactamases had been identified. Since then, evolution has

escalated: today more than 1300 different beta-lactamase variants exist. The emergence of ESBL, AmpC and carbapenemases just within the two last decades has been rapid. All these enzymes have also been detected in bacteria of animal origin, but later and fewer than in bacteria of human origin. Aminopenicillins can select narrow-spectrum beta-lactamases, like penicillinases and TEM-1 type beta-lactamases. The use of extended-spectrum cephalosporins especially, and later carbapenems, is considered to be one of the main reasons for recent emergence of extended spectrum beta-lactamases and carbapenemases in clinically-relevant bacteria, such as *Escherichia coli*, *Klebsiella* spp. and *Salmonella* spp. The wide use of beta-lactam inhibitor combinations to combat emerging penicillinases is also considered to be a driving force favouring the evolution and emergence of inhibitor-resistant AmpC type beta-lactamases (Bush, 2013).

538 **Table 1.** Examples of the most clinically relevant beta-lactamases, their target antimicrobials and
539 bacterial families or genera where present. The group classification is based on Bush and Jacoby's
540 classification of beta-lactamases.

Group	Examples of enzyme families	Antimicrobial targets /cross-resistance*	Ambler class	Examples of bacterial families/genera where described
Group 1 cephalosporinases (AmpC-type)	CMY, ACT, DHA, FOX, MIR	Natural penicillins, aminopenicillins and their inhibitor combinations 1-3rd-gen. cephalosporins cefamycins	C	Enterobacteriaceae *Acinetobacter* spp.** *Pseudomonas* spp.**
Group 2 serine beta-lactamases	Penicillinases	Natural penicillins, amino-penicillins	A	*Staphylococcus* spp.
	TEM-1, TEM-2, SHV-1, ROB-1, BRO	Natural penicillins aminopenicillins 1st –gen. cephalosporins	A	Enterobacteriaceae *Neisseria* spp. Pasteurellaceae (ROB-1) *Moraxella* spp. (BRO)
	ESBL-variants of the TEM, SHV, CTX-M	Natural penicillins, amino-penicillins, 1-4th-gen. cephalosporins, monobactams	A	Enterobacteriaceae
	KPC, carbapenemase variants of SHV	Natural penicillins, amino-penicillins (some enzymes also destroy their inhibitor combinations), 1-4th-gen. cephalosporins, monobactams, carbapenems	A	Enterobacteriaceae *KPC also in Acinetobacter spp.* and *Pseudomonas aeruginosa*
	Carbapenemase variants of OXA (oxacillinases)	Natural penicillins, amino-penicillins and their inhibitor combinations staphylococcal penicillins, 1st -gen. cephalosporins, carbapenems (low level)	D	*Pseudomonas aeruginosa, Acinetobacter* spp. Enterobacteriaceae
Group 3 carbapenemases (metallo-beta-lactamases)	IMP, VIM, NDM	Natural penicillins, aminopenicillins and their inhibitor combinations, 1-4th gen.cephalosporins, cefamycins, carbapenems	B	Enterobacteriaceae *Pseudomonas aeruginosa Acinetobacter* spp.

541 * There is substantial variation in how different enzymes or their variants hydrolyse different drugs. For example,
542 CTX-M type enzymes are more effective at destroying cefotaxime, while SHV-type ESBL-enzymes destroy
543 ceftazidime better. In addition there can be variation in inhibitor (clavulanic acid, tazobactam, sulbactam)
544 resistance.

545 ** Inducible chromosomal AmpC beta-lactamases are important resistance mechanisms in these.

546

3.1.2. Modification of the target site

Another important mechanism of beta-lactam resistance is alterations in penicillin binding proteins, PBPs. This type of mechanism is common in staphylococci and is mediated by *mec*A or *mec*C genes (Feng et al., 2008; García-Álvarez et al., 2011). Recently the presence of another *mec*-gene, *mec*B (*mec*A_m), was verified in a human *Staphylococcus aureus* isolate (Becker et al., 2018; Gómez-Sanz et al., 2015). The result of the *mec*-gene is a modified penicillin binding protein with low affinity to nearly all beta-lactams except to the staphylococcal cephalosporins, ceftobiprole and ceftaroline. *mec* gene-harbouring staphylococci are known as methicillin-resistant staphylococci (MRS). Today, methicillin resistance is a common feature in *Staphylococcus aureus*, *Staphylococcus pseudintermedius* and in many coagulase negative staphylococci (Hanssen and Ericson Sollid, 2006). The origin of the *mec*B gene is in Macrococcus species of animal origin (Baba et al., 2009). Macrococcus spp. can also harbour the fourth mec variant, *mec*D (Schwendener et al., 2017). The *mec* genes locate in a chromosomal genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). To date twelve different SCC*mec* elements have been described with several subtypes (www.sccmec.org). There is evidence suggesting that *mec* genes or SCC*mec* elements are transferrable between different staphylococcal species (Bloemendaal et al., 2010; Hanssen and Ericson Sollid, 2006). mecB can also be plasmid encoded (Becker et al., 2018). Methicillin-resistant staphylococci can also spread clonally.

Modification of PBPs is a cause of beta-lactam resistance in *Streptococcus* spp., *Enterococcus* spp., *Neisseria* spp. and *Haemophilus* spp., although the genes conferring resistance are dependent on the bacterial species in question (Zapun et al., 2017). In streptococci, enterococci and *Haemophilus* spp., alterations in PBPs cause gradually decreasing susceptibility to the beta-lactam in question. This type of resistance is mediated by mutations and genetic recombination of PBP-encoding genes. The level of cross resistance to other beta-lactam(s) depends on the PBP mutation in question, the antimicrobial substance and general PBP composition of the bacterium. Apart from horizontal transfer of the genetic material within the same or closely related bacterial species, the resistance is also spread clonally. There are differences between bacterial species in genetic recombination rate (Zapun et al., 2017).

3.1.3. Other resistance mechanisms

A third mechanism of beta-lactam resistance is decreased expression of outer membrane proteins. To access PBPs on the inner plasma membrane of Gram-negative bacteria, beta-lactams must diffuse or use the porin channels of the outer membrane to enter the bacterial cell. There may be loss of porin channels, or changes in their structure due to mutations, that result in lower permeability to beta-lactams. Another mechanism of beta-lactam resistance is due to non-selective multi-drug efflux pumps (either acquired or intrinsic) which remove a wide range of substrates from the periplasmic space to the surrounding environment. These types of pumps exist commonly in Gram-negative species. Bacteria can have simultaneous porin channel changes and efflux-pumps together with beta-lactamase genes which may result in an odd or misleading resistance phenotype (Tang et al., 2014).

3.2. Susceptibility testing

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical Laboratory Standards Institute (CLSI) have guidelines for the susceptibility testing of bacterial species against ampicillin, amoxicillin and/or amoxicillin-clavulanic acid, but there are variations in methodologies and breakpoints between these two. Susceptibility testing can be performed manually, but there are also semi-automated or automated systems on the market (Reller et al., 2009). From a technical point of

view it should be noted that beta-lactam solutions are not very stable. They can lose their activity even when frozen, although ampicillin maintains its activity better than amoxicillin (Okerman et al., 2007). Clavulanic acid is chemically unstable, and moisture, temperature and pH affect its degradation (Saudagar et al., 2008). These challenges can be controlled with a proper quality control scheme.

Both EUCAST and CLSI have breakpoints for human pathogens, while CLSI has ampicillin breakpoints for certain swine, horse and canine pathogens, and breakpoints for amoxicillin-clavulanic acid for some canine and feline pathogens. For ampicillin and amoxicillin-clavulanic acid, breakpoints for canine and feline urinary tract infection pathogens are higher than for other infection sites. The majority of CLSI's veterinary specific breakpoints are only for the dilution method (CLSI, 2015). If no veterinary specific breakpoints are available, breakpoints for human pathogens are applied to interpret susceptibilities of veterinary bacterial isolates.

EUCAST has published epidemiological cut-off (ECOFF) values for ampicillin and amoxicillin for several bacterial species, but none yet for the combination of amoxicillin-clavulanic-acid, even though MIC and zone distributions are available for many species (www.eucast.org). ECOFFs, when available, are used by EFSA for EU-wide indicator and zoonotic bacteria resistance surveillance. EUCAST has no clinical breakpoints for veterinary pathogens but a subcommittee of the EUCAST – VetCAST - was founded in 2015 aiming to contribute to global standards for susceptibility testing and setting breakpoints for different bacterial species of animal origin. (http://www.eucast.org/ast_of_veterinary_pathogens/).

Ampicillin susceptibility is commonly used as a surrogate for amoxicillin susceptibility, except for the amoxicillin-clavulanic acid combination. EUCAST uses a fixed, 2 mg/L, clavulanic acid concentration in each dilution when testing MICs for amoxicillin-clavulanic acid while CLSI uses a 2:1 ratio of amoxicillin to clavulanic (Díez-Aguilar et al., 2015). Ampicillin or amoxicillin-clavulanic acid susceptibility results do not provide information on whether the bacterial isolate in question produces broad spectrum beta-lactamases (ESBL/AmpC/CPE). Therefore it is vital that testing panels in veterinary laboratories include antimicrobials that facilitate the recognition of bacterial isolates that may have reduced susceptibility to 3rd-generation cephalosporins or carbapenems even though testing would not be necessary for clinical purposes. There are several phenotypic and genotypic methods available to identify the type of beta-lactamase in suspected isolates either in clinical or reference laboratories (Decousser et al., 2017).

For some other bacterial species, screening of beta-lactam resistance using a surrogate is preferred over direct testing of particular drugs. For example, for screening for penicillin non-susceptibility in *Streptococcus pneumoniae*, an oxacillin disk is used. If reduced susceptibility is observed, then MIC determinations for clinically relevant beta-lactams are performed. Otherwise the isolate can be interpreted as susceptible for beta-lactams without further testing (Jetté and Sinave, 1999). Another example is *Haemophilus* spp. and *Histophilus* spp., in which resistance to beta-lactams can be due to beta-lactamases or changes in PBPs: the former can be screened with a beta-lactamase test and the latter by using either a low potency ampicillin or benzyl penicillin disk (Skaare et al., 2015). Beta-lactamase tests are also used for other bacterial species such as staphylococci. There are several types of beta-lactamase tests available. Depending on bacterial species, phenotypic tests have some limitations in sensitivity. Therefore in severe infections genotypic tests are preferred (Jenkins and Schuetz, 2012). The method used to screen for *mec*A/C-mediated methicillin resistance in staphylococci (and thus resistance to all beta-lactams except for 5th-generation cephalosporins) depends on the staphylococcal species: for example, cefoxitin disk is the drug of choice to screen for methicillin resistant *S. aureus* (MRSA) while oxacillin is the drug of choice for screening in methicillin resistant *S. pseudintermedius* (MRSP) (Wu et al., 2016).

4. Sales and use of aminopenicillins and their inhibitor combinations in veterinary medicine

4.1. Sales

Measured in mg/PCU, penicillins [extended spectrum penicillins (ampicillin, amoxicillin), beta-lactamase sensitive penicillin (benzyl penicillin, penethamate, phenoxymethylpenicillin) and beta-lactamase-resistant penicillins (cloxacillin, dicloxacillin)] were the second most sold antimicrobial class in food animal species in the EU in 2015 and accounted for 25% of the total antimicrobial sales (EMA/ESVAC, 2017). Geographically, Spain and Italy had the highest relative sales of penicillins, while Sweden, Norway and Italy the lowest (Figure 1). Extended spectrum penicillins (ampicillin, amoxicillin, and their inhibitor combinations) made up the major proportion (88%, 30.0 mg/PCU) of the total use of penicillins (Figure 2), although wide variation between the member states was observed. There were only six European countries (Denmark, Finland. Iceland, Luxembourg, Norway, Sweden) in which beta-lactamase sensitive penicillins (benzyl penicillin, penethamate, phenoxymethylpenicillin) contributed more than half of the total beta-lactam sales, while in 23 out of 30 countries, amoxicillin and ampicillin consumption contributed more than half of the total penicillin sales. Aminopenicillins and their inhibitor combinations formed a very limited fraction of the total sales of aminopenicillins both at the European level (1%, 0.3 mg/PCU) and by country (Figure 2 and Figure 3). The majority of sales of amoxicillin-clavulanic acid combinations was as tablets (EMA/ESVAC, 2017). According to the ESVAC 2015 data, total sales of veterinary authorised tablets containing extended spectrum beta-lactams was 25 tonnes, of which 88% were beta-lactams and their inhibitor combinations (Figure 4). In only five countries the sales of extended spectrum beta-lactams without an inhibitor dominated (Figure 5). It can be assumed that tablets are mainly used for to treat infections in dogs and cats.

4.2. Use and indications in food-producing animals

Ampicillin, amoxicillin, and to a lesser extent, amoxicillin-clavulanic acid combinations, have been widely used for decades for the treatment of infections in food-producing animals in the EU. There are numerous aminopenicillin products with several indications available for cattle, pigs, and poultry in EU countries, for parenteral, oral, intrauterine or intramammary administration. In addition to the treatment of infections in various organs, metaphylactic or prophylactic indications are included in SPCs. For products containing amoxicillin-clavulanic acid, the spectrum of different indications is narrower compared to ampicillin and amoxicillin products, but is still wide. The target pathogens include genera with variable inherent susceptibility such as *Actinobacillus* spp., *Pasteurella* spp., *Bibersteinia* spp., *Haemophilus* spp., *Histophilus* spp., *Mannheimia spp.*, *Streptococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *Moraxella* spp., *Trueperella* spp., *Erysipelothrix* spp., *Clostridium* spp., *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Bordetella bronchiseptica* and *Aeromonas salmonicida*. Of these, the five last mentioned are inherently less susceptible to aminopenicillins compared to other genera.

The recommended dosages are variable ranging from 5 - 20 mg/kg Q 6 - 48 hrs depending on product, its chemical formula, the method of administration and animal species. Oral administration is possible only to monogastric animals or young calves and foals before maturation of the GI-tract (Giguère et al., 2013). In general, the recommended duration of the treatment with orally administered products (premixes, drinking water formulations, oral boluses) ranges from 3 to 15 days and with injectables usually from 3 - 5 days. Information on target animal species, drug formulas, indications (including target pathogens) and treatment durations are summarised in Table 5 and is based on examples that

have been collected from SPCs of the veterinary authorised products available in the UK, France, Spain and Germany.

In pigs aminopenicillins are authorised for the treatment of respiratory infections, GI-tract infections, meningitis, arthritis, and skin and soft tissue infections. With premixes the duration of treatment is up to 15 days and with drinking water formulations up to 5 days. With injectables the recommended treatment duration is 3 - 5 days. In cattle, indications include, among others, respiratory tract infections, GI-tract infections, soft tissue infections and urogenital infections. In calves amoxicillin has an indication to be used as oral bolus for the treatment of umbilical cord infections and enteritis. In cattle aminopenicillins are also authorised for the treatment of sub-clinical and clinical mastitis. In intramammary formulations, an aminopenicillin is combined with an anti-staphylococcal penicillin, although the amoxicillin-clavulanate combination is also available. The indications for sheep and other ruminants are the same as for cattle, although marketing authorisations seldom cover other ruminant species. For poultry, indications include respiratory and GI-tract infections. Amoxicillin is also authorised for the treatment of furunculosis caused by *A. salmonicida* in Atlantic salmon, administered as a top dressing mixed in fish feed for 10 days.

4.3. Use and indications in horses

Although rarely investigated, there is evidence that benzyl penicillin - with or without procaine - is the most frequently used beta-lactam in equine medicine in the EU (De Briyne et al., 2014; Hughes et al., 2013; Thomson, 2010). Aminopenicillins, mainly ampicillin, have been mentioned in the textbooks as an option for treating various equine infections (Weese et al., 2008). There is at least one amoxicillin sodium containing product (in Germany) and an ampicillin sodium product (in Ireland and UK) that have authorization for horses, but no definite information exists whether there are other equine authorised aminopenicillin products in the EU. Therefore human authorised intra-venous ampicillin formulations are used off-label in horses according to the Cascade principle (Keith Baptiste, oral communication). There are no estimates of the volume of aminopenicillin – or any other antimicrobial – consumption in horses at the EU level, but according to some national reports, antimicrobial consumption of horses in general contributes only a small proportion of total veterinary antimicrobial consumption (DANMAP, 2016; SDa, 2017). Due to relatively few authorised antimicrobial products and due to regional differences in availability of authorised products, off label use of antimicrobials in horses is common. The horse is regarded as a food-producing animal species unless declared as not being intended for slaughter for human consumption in accordance with Commission decisions 93/623/EEC and 2000/68/EC. Non-food-producing horses can be treated with a far wider range of antimicrobials. It is therefore possible, that other extended spectrum penicillin classes are also used for horses, but there are no data available about such use.

Target pathogens for aminopenicillins in horses include streptococci, enterococci, *Pasteurellaceae* (incl. *Actinobacillus*), *Listeria* spp., and Enterobacteriaceae (including *Salmonella* spp.) in various organ systems. Aminopenicillins may be combined with an aminoglycoside when treating neonatal infections or severe polymicrobial infections in adult horses (Weese et al., 2008). Aminopenicillins cannot be administered to adult horses orally due to their poor absorption from GI-tract and the risk for antimicrobial associated diarrhoea. Therefore the most common route is intramuscular or intravenous injection. Ampicillin sodium is the preferred formulation since intra-muscular injection of amoxicillin or ampicillin trihydrate results in low drug concentrations in plasma (Brown et al., 1982; Haggett and Wilson, 2008). Amoxicillin trihydrate can also cause tissue irritation (Haggett and Wilson, 2008). The recommended dose range for ampicillin sodium is 10 - 40 mg/kg i.v. TID-QID or 10 - 22 mg/kg BID

i.m. A PK/PD simulation leads to the conclusion that with a dosage of 10 mg/kg i.m. BID-TID most streptococcal infections would be treated successfully, but for staphylococcal infections, dosages of 15 mg/kg i.m. QID would be needed to achieve sufficient T>MIC. As this would result in a high volume to be injected, intravenous treatment is often a more practical and ethical option (Hoven et al., 2003).

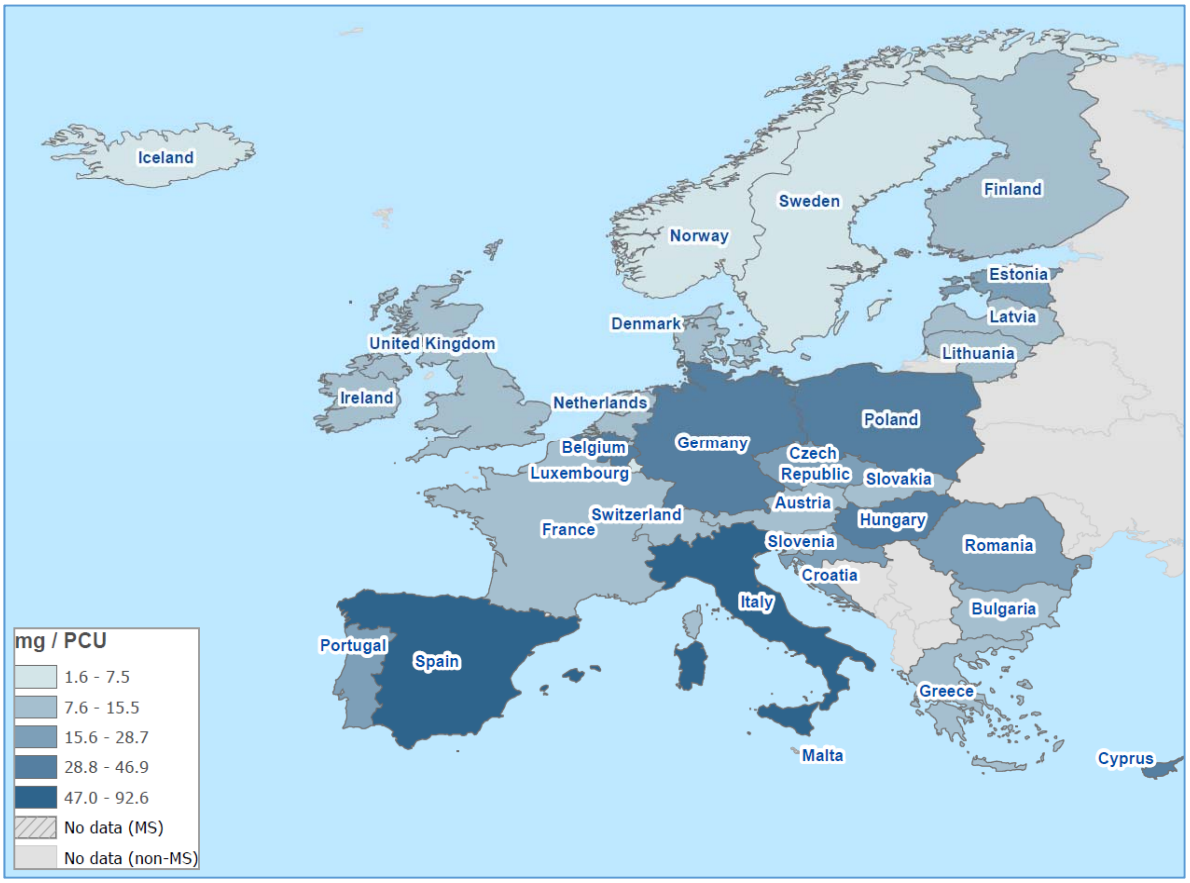
4.4. Use and indications in companion animals

In dogs and cats beta-lactams are probably the most commonly used antimicrobials, with special reference to aminopenicillins and their inhibitor combinations (Holso et al., 2005; Radford et al., 2011; Rantala et al., 2004), although there is lack of systematic data collection for these species. Of veterinary authorized tablets containing extended spectrum penicillins, beta-lactamase inhibitor combinations were the most sold agents (EMA/ESVAC, 2017).

Infections treated with aminopenicillins in dogs and cats include respiratory tract infections, urinary tract infections, genital infections, wound infections, skin and soft tissue infections, and enteric conditions (Rantala et al., 2004). A wide range of Gram-positive and Gram-negative bacterial species are mentioned as target pathogens in SPCs of aminopenicillin products, such as staphylococci, streptococci, *Pasteurella* spp., *Clostridium* spp., *Proteus* spp., *E. coli,* and *B. bronchiseptica*. Suggested treatment periods range from 5 days to several weeks for tablet formulations depending on whether the condition is acute or chronic, and for injectables usually from 3 to 5 days. The common dosage range is from 10 mg/kg up to 25 mg/kg for tablets. Apart from veterinary authorised products, human authorised products – especially those intended for intravenous use - are used to treat companion animal infections, but data about the extent of such use are not readily available. Cascade use may include also other extended spectrum penicillins. The use of human- authorised intravenous amoxicillin-clavulanic acid has been associated with hypersensitivity-type side effects in companion animals, but these are possibly related to components other than the antimicrobial substances (Rollin et al., 1986; Willard et al., 1998).

745

746 **Figure 1.** Distribution of sales of penicillins (ATC J01C) for veterinary use in mg/PCU, in 30 European
747 countries, for 2015. Source: ESVAC



748
749

Figure 2. Proportion of average sales of veterinary authorised penicillins in mg/PCU by subclass in the European countries, 2015, Source: ESVAC

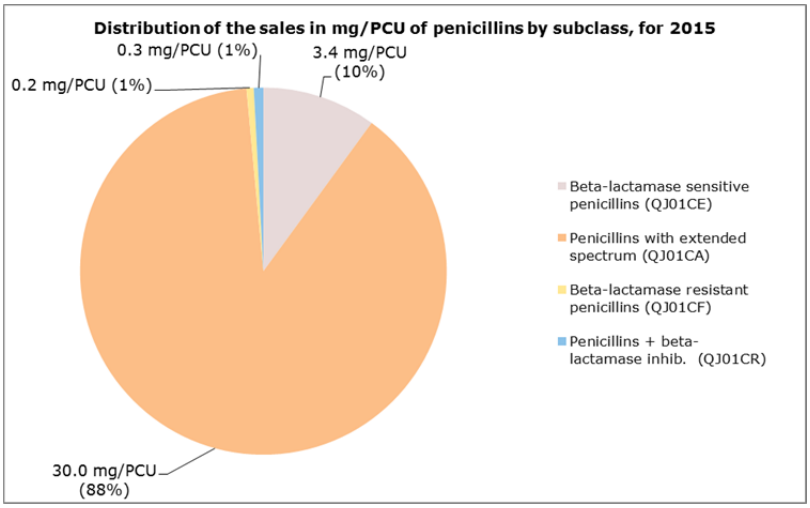


Figure 3. Proportional sales of veterinary authorised beta-lactams by country in 30 European countries. The figure includes penicillins with extended spectrum (ampicillin, amoxicillin), beta-lactamase sensitive penicillin (benzyl penicillin, penethamate, phenoxymethylpenicillin) and beta-lactamase- resistant penicillins (cloxacillin, dicloxacillin) Source: ESVAC

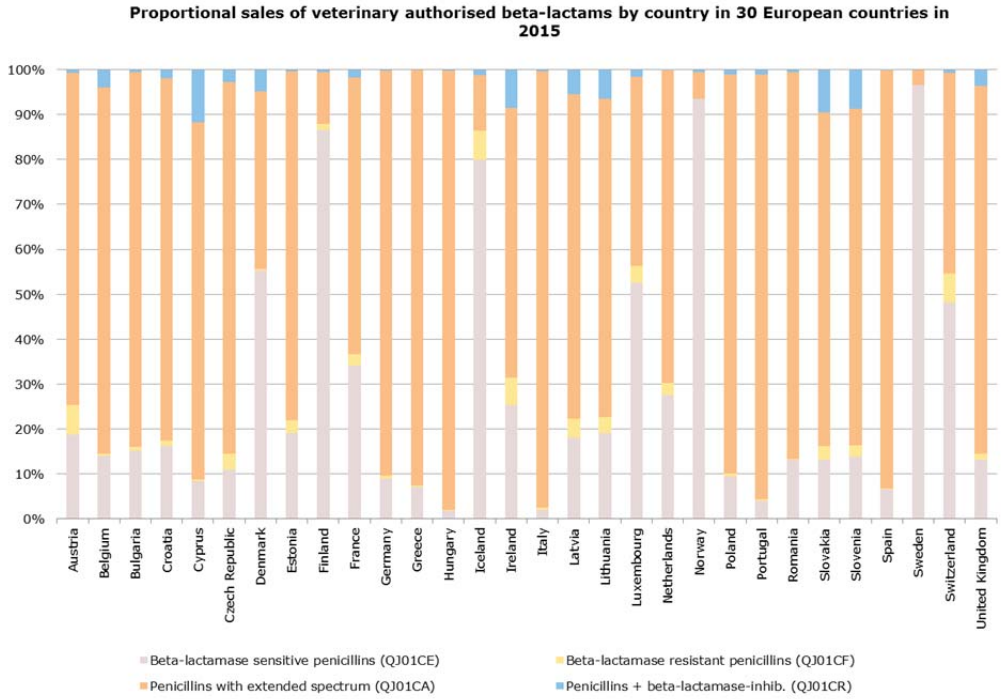


Figure 4. Proportion of sales of veterinary authorised tablets containing extended spectrum penicillins and their inhibitor combinations summed by 30 European countries in 2015. Calculated by weight of active ingredient. Source: ESVAC

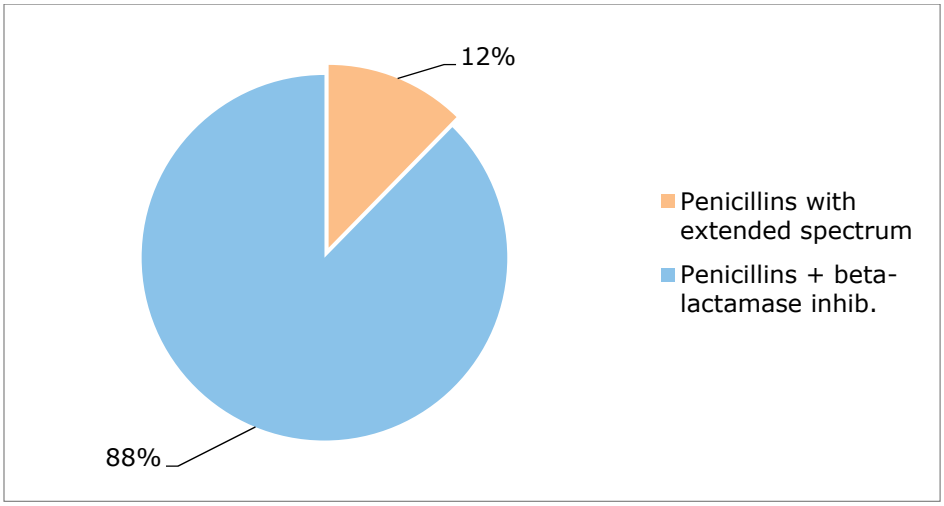
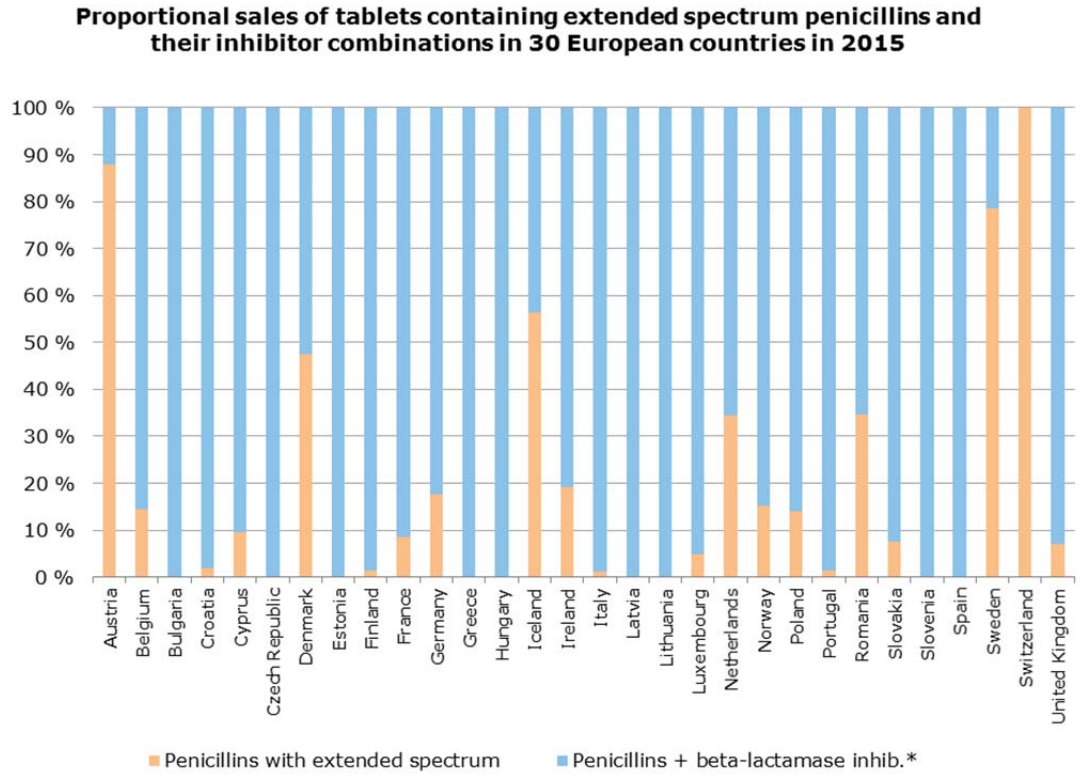


Figure 5. Proportional sales of veterinary authorised tablets containing extended spectrum penicillins and their inhibitor combinations by country in 30 European countries in 2015. Proportions calculated by weight of active ingredient. Source: ESVAC



5. The use of aminopenicillins and their inhibitor combinations in human medicine

This chapter reviews the indications and use of aminopenicillins and their inhibitor combinations in human medicine. As the focus of this paper is on substances that have veterinary authorisation, only indications relevant to ampicillin, amoxicillin and their amoxicillin clavulanic-acid inhibitor are covered while it is acknowledged that sales figures include also other extended spectrum penicillins and other inhibitor combinations.

5.1. Indications in human medicine

The aminopenicillins are important antimicrobials that have a broad spectrum of activity. They and their inhibitor combinations (e.g. amoxicillin-clavulanic acid) have been classified by the WHO as critically important antimicrobials for human medicine because there is high frequency of use of aminopenicillins in human medicine and there are limited therapeutic options for infections caused by *Listeria monocytogenes* and *Enterococcus* spp (). In addition, there is a possibility of transmission of resistant bacteria or resistance genes from non-human sources to humans (WHO, 2017). Ampicillin, amoxicillin and the amoxicillin-clavulanic acid combination are included in the WHO Model List of Essential Medicines in the "access" group as first and second choice antimicrobials for the empiric treatment of most common infectious syndromes including community and hospital acquired pneumonia, bacterial pharyngitis, sinusitis, otitis media, sepsis in neonates, lower UTI, acute bacterial meningitis (when listeriosis is suspected), intra-abdominal infections, and skin and soft tissue infections (http://www.who.int/medicines/publications/essentialmedicines/en/).

Apart from infections caused by *L. monocytogenes* and enterococci, aminopenicillins and their beta-lactamase inhibitors are among the first-line therapy for the treatment of upper and lower respiratory tract infections, such as community-acquired pneumonia, otitis media and bacterial sinusitis caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* (Harris et al., 2016; Lee et al., 2015; McCulloh and Patel, 2016; Schilder et al., 2017; Woodhead et al., 2005), pharyngitis and other infections caused by (group A, B, C and G) beta-haemolytic streptococci (Sidell and L Shapiro, 2012; Wessels, 2016) and skin and soft tissue infections caused by other susceptible organisms (Brook, 2016; Jacobs et al., 2007). Aminopenicillins can also be used for infections caused by *E. coli* (mainly urinary tract infections) and *Neisseria gonorrhoeae*, provided that they are caused by susceptible organisms (Stein et al., 2015). Amoxicillin is used in combination with clarithromycin and metronidazole to eradicate *Helicobacter pylori* (Qasim et al., 2009).

Low-level resistance caused by target site modification (PBPs) in *Streptococcus pneumoniae* can be managed by elevating the dose of an aminopenicillin (Jacobs, 2008). Emergence of beta-lactamase-mediated resistance in common pathogens limits the usefulness of aminopenicillins. When combined with an inhibitor, their activity can be maintained, provided that the beta-lactamase in question does not hydrolyse the inhibitor. For example, due to the frequency of beta-lactamase mediated resistance in *Haemophilus influenzae* and *Moraxella catarrhalis*, infections caused by these organisms are often treated with an inhibitor combination. Clinical trials have supported a high clinical efficacy of amoxicillin-clavulanic acid in the treatment of respiratory infections, urinary tract infections, skin- and soft tissue infections, intra-abdominal infections, as well as obstetric and gynaecological infections caused by the target organisms. It is also effective for mixed infections where anaerobes can be present (Ball, 2007). Amoxicillin-clavulanic acid is recommended as the first line treatment for canine and feline bite wound infections in humans (Ellis and Ellis, 2014; Esposito et al., 2013). Some studies

have suggested that amoxicillin-clavulanic acid could be an option to treat infections caused by ESBL-producing Enterobacteriaceae, provided that the pathogen is susceptible to it and that high doses are given at frequent intervals (Beytur et al., 2015; Rodríguez-Baño et al., 2011).

Table 2. Aminopenicillins including their inhibitor combinations that fulfil WHO criterion 1 with comments addressing EU concerns.

Antimicrobial class	Bacterial targets in human medicine (for which availability of class/substance is critically important due to few alternatives)	Relative frequency of use in humans in the EU	Antimicrobial class
Penicillins: Aminopenicillins including β-lactamase inhibitors combinations (e.g. amoxicillin + clavulanic acid)	*Listeria* spp. *Enterococcus* spp.	Amoxicillin and their inhibitor combinations are the most used penicillins in the EU in humans, although mainly to other indications than infections caused by these bacteria. The exact consumption figures are not available, since they are reported as a group level in humans.	*Enterococcus* spp. Enterobacteriaceae

5.2. Consumption of aminopenicillins in humans in the EU

According to the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) summary report on 2016 data, the most commonly used antimicrobials in human medicine were penicillins (ATC J01C), ranging from 33% (Germany) to 67% (Slovenia) of the total antimicrobial consumption in the community. Total use of penicillins (J01C) tended to be high in countries with a high total use of antibiotics and vice versa. The highest penicillin use (DDD per 1000 inhabitants and per day) was observed in France, Belgium, Italy and Ireland, and the lowest in Estonia and the Netherlands (Figure 6). Penicillins are also frequently used in hospitals, although the use of cephalosporins and other beta-lactams, including carbapenems (J01D), dominates in the hospital hospital sector (ECDC, 2017b). Data that specify human use of aminopenicillins (J01CA01, J01CA04) and inhibitor combinations (J01CR02) that are also authorised for veterinary use are not readily available. The antimicrobial consumption database, ESAC-Net (https://ecdc.europa.eu/en/antimicrobial-consumption/surveillance-and-disease-data/database), reports consumption data on extended-spectrum penicillins at the J01CA and combinations of penicillins, including beta-lactamase inhibitors at the J01CR group levels, respectively. Both these groups include several other substances that are not used in animals (Appendix, Table A1).

In 2016, for the J01CA group, consumption in the community ranged from 0.2 (Malta) to 12.2 (France) Defined Daily Doses (DDDs) per 1000 inhabitants per day, while the respective range in hospital sector was from 0.023 (Bulgaria) to 0.751 (Lithuania) DDDs. Regarding the consumption of group J01CR

substances in the community, the highest use was observed in Italy and the lowest in Norway (11.7 vs. 0.0 DDDs/1000 inhabitants/day, respectively). In hospitals, the highest consumption of J01CR class drugs was in Slovakia and the lowest in Norway (0.809 vs. 0.079 DDDs/1000 inhabitants/day, respectively).

According to the latest EU surveillance report on antimicrobial consumption, consumption of amoxicillin (J01CA04) ranged from 0.9 (Sweden) to 9.7 (France) DDD per 1000 inhabitants per day, while consumption of amoxicillin with an enzyme inhibitor (J01CR02) ranged from 0.003 (Norway) to 10.3 (Italy) DDD per 1000 inhabitants per day. Amoxicillin - alone or in combination with an enzyme inhibitor - was the most used antibacterial agent in human medicine in as many as 22 of 30 EU/EEA countries, with the exception of Denmark, Norway and Sweden, where the most frequently used penicillin was phenoxymethylpenicillin (ECDC, 2014). In seven countries (Bulgaria, Croatia, Italy, Luxembourg, Malta, Portugal and Slovakia) the J01CR group accounted for ≥ 75% of the total consumption of penicillins (J01C). A significant increase was detected in the consumption of group J01CR drugs in ten countries (Austria, Denmark, Estonia, France, Germany, Ireland, Italy, Luxembourg, Slovenia and the United Kingdom), and no countries reported a decrease in consumption of these substances. Furthermore, the consumption of extended-spectrum penicillins and their inhibitor combinations (J01CA and J01CR) during 2008-2011 showed an increasing trend, as well as for the consumption in all EU/EEA countries while the use of beta-lactamase-sensitive penicillins (ATC group J01CE) decreased significantly (ECDC, 2014).

If human and animal usage of penicillin and other beta-lactams are compared as mg/kg of estimated biomass, human use is approximately twice that for animals in EU/EEA countries (80 vs. 40 mg/of estimated biomass) (ECDC/EFSA/EMA, 2017).

Figure 6. Use of penicillins (ATC J01C) in humans (DDD per 1000 inhabitants and per day) in the community in European countries in 2015. Data for Cyprus and Romania are reported jointly for community and hospital, and therefore are not included in this map. Bulgaria, Hungary and Slovakia reported no consumption figures for beta-lactamase resistant penicillins in 2015. Source: ESAC-Net

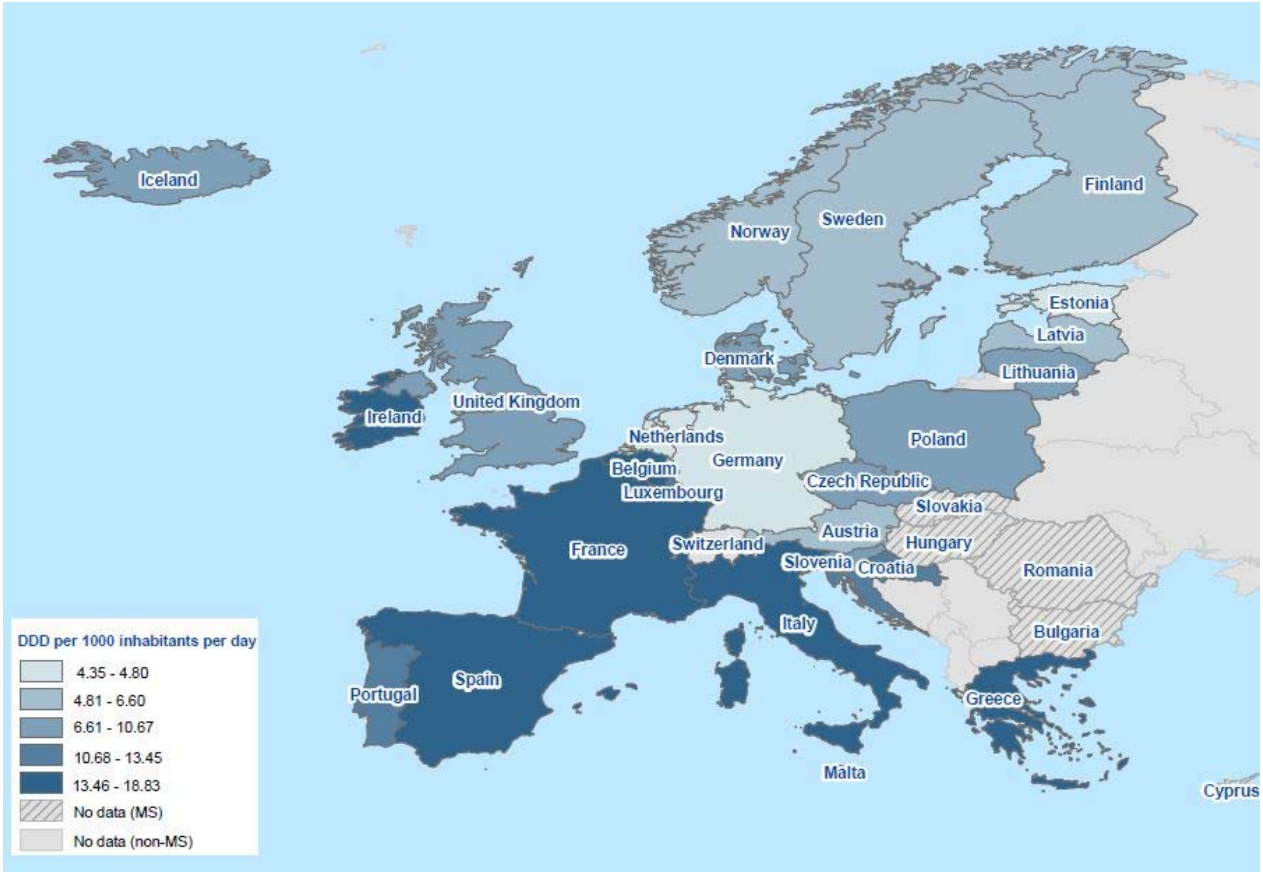
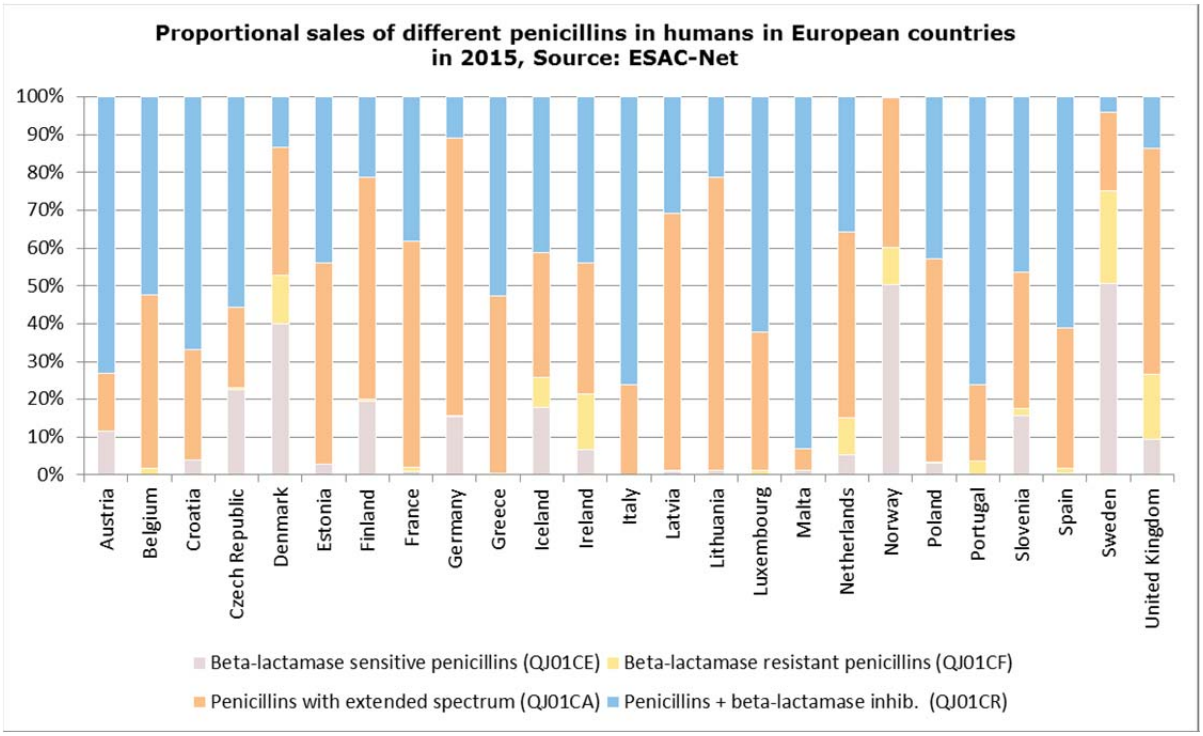


Figure 7. Proportional sales of different penicillins in community in humans in European countries in 2015. Data for Cyprus and Romania are reported jointly for community and hospital and are thus not included into this graph. Bulgaria, Hungary and Slovakia reported no consumption figures for beta-lactamase resistant penicillins in 2015. Source: ESAC-Net.



6. Occurrence of resistance

This section summarises the occurrence of aminopenicillin resistance in veterinary and human bacteria. In addition to aminopenicillin resistance, the existence of resistance to other beta-lactams that could be co-selected by the use of aminopenicillins is reviewed. First, an overview of zoonotic, indicator and other bacteria covered by EU wide surveillance is given. Then resistance in certain animal pathogens is summarised. Since animal pathogens are not included in EU wide surveillance, the resistance data regarding animal pathogens are only examples based on scientific publications or national surveillance reports. Due to differences in methodology, target population and breakpoints, the purpose is not to give comparable data, but to give a rough overview of the occurrence of aminopenicillin resistance in some major animal pathogens for certain animal species (pigs, cattle, poultry, dogs and cats). In the last section of this chapter, resistance in some human pathogens in Europe is viewed.

In general Group A, B, C, G streptococci (beta-haemolytic streptococci) should be considered by default susceptible to penicillin and aminopenicillins regardless of bacterial species or its origin (animal/human). Aminopenicillin (or penicillin) resistance in clinical *L. monocytogenes* is very rare, but has been described in food or environmental isolates (Lungu et al., 2011). Also penicillin/aminopenicillin MICs for *Erysipelothrix rhusiopathiae* are very low (Eriksson et al., 2009). Therefore these bacterial species are not addressed in this section.

6.1. Resistance in bacteria of animal origin covered by EU surveillance

Resistance to aminopenicillins is very frequent in indicator *E. coli* of food-producing animals or in meat of animal origin with the EU mean ranging from 31.0% (for cattle) to 64.6% (for turkeys) (Table 3). Statistics also indicate large variation between the countries. A multi-drug resistance (MDR) profile in ampicillin-resistant *E. coli* is very common. Apart from ampicillin resistance, MDR *E. coli* are often simultaneously resistant to fluoroquinolones, sulphonamides, and tetracycline (EFSA/ECDC, 2014, EFSA/ECDC, 2016). Ampicillin resistance in *Salmonella* spp. ranges from nearly 4.1% (laying hens) to 44.7% (turkeys) in the EU with wide variation between the countries (from 0 to 87.5%). There is also variation in resistance between different salmonella serovars (Table 4; EFSA/ECDC, 2014, EFSA/ECDC, 2016). The occurrence of ESBL-/AmpC-producers in *Salmonella* spp. and indicator *E. coli* from poultry is uncommon (EFSA/ECDC, 2016). It should be noted, however, that indicator bacteria resistance figures are based on susceptibility testing of random bacterial colonies from non-selective media. In the case of specific ESBL/AmpC/carbapenemase monitoring, in which pre-enrichment and selective plating of specimens are used, the occurrence of ESBL/AmpC *E.coli* has been detected as very high in fattening turkeys (42%), broilers (47%), and in broiler meat (57%) with both wide variation in enzyme types as well as total occurrence between the countries (reference: EFSA/ECDC 2018).

The latest results from mandatory monitoring for indicator enterococci are from the year 2013. Ampicillin resistance rates were moderate (EU mean 22.7%) in *Enterococcus faecium* from broilers. For other animal species, ampicillin resistance in this bacterium ranged from 0 - 11.2%, being highest in breeding pigs (9%) and young cattle (11.2%) in Belgium. Ampicillin resistance was not observed in indicator *Enterococcus faecalis* from broilers, fattening pigs, breeding pigs, or in bovine species. Only Norway reported approximately 1% ampicillin resistance in laying hen flocks for this bacterial species (EFSA/ECDC, 2015). Regarding enterococci from meat, ampicillin resistance was absent in *E. faecalis* and *E. faecium* from porcine or bovine meat and was very low (0.3%) in *E. faecalis* of broiler meat origin. *E. faecium* isolates from meat of porcine or bovine origin were all susceptible to ampicillin, but on average, 7.6% of *E. faecium* isolates from broiler meat specimens showed ampicillin resistance with a range of 1.5 – 13.3% by countries (EFSA/ECDC, 2015). It should be noted that these figures are based on a low number of isolates from only a few Member States (MSs).

In an EFSA/ECDC report concerning the year 2014, seven EU/EFTA countries reported monitoring results for MRSA in food-producing animals or their environment and six countries reported results for MRSA in food of animal origin. In dairy cows, MRSA rates were 9.7% (Germany) and 16.9% (Netherlands), in pigs 0 – 60% (Iceland, Norway, Switzerland, Netherlands) and in turkeys 21.9% (Germany). MRSA was observed in meat from broilers (Switzerland), turkeys (Germany) and pigs (Spain) with a range of 3.2 - 42.5% positive batches, being the highest in turkey meat (EFSA/ECDC, 2016). The data is not comparable between the countries or even animal species within a country due to differences in sampling methods and target populations.

928 **Table 3.** Ampicillin resistance (ECOFF > 8 mg/L) in indicator *Escherichia coli* (ECOFF > 8 mg/L)
929 according to EFSA reports on AMR monitoring (EFSA/ECDC, 2017; EFSA/ECDC, 2018)

	Ampicillin resistance % (number of tested isolates) in indicator *E. coli*			
	Broilers 2016	Turkeys 2016	Pigs 2015	Cattle 2015
EU mean value	58 (4,729)	64.6 (1,714)	39.3 (4 268)	31.0 (1 734)
Maximum country value	100 (1000)	85.9 (170)	89.1 (55)	61.2 (170)
Minimum country value	8.7 (184)	8.2 (85)	12.9 (163)	1.4 (74)
No. of countries providing data	27	11	27	10

930
931
932 **Table 4.** Ampicillin resistance (ECOFF > 8 mg/L) in *Salmonella* spp. according to EFSA reports on AMR
933 monitoring (EFSA/ECDC, 2017; EFSA/ECDC, 2018)

	Ampicillin resistance % (number of tested isolates) in *Salmonella* spp.				
	Broilers 2016	Laying hens 2016	Turkeys 2016	Pigs* 2015	Cattle** 2015
EU mean value	17.1 (1,717)	4.1 (1,216)	38.3 (663)	44.7 (750)	40.0 (80)
Maximum country value	48 (25)	18.1 (11)	77.8 (27)	87.5 (8)	66.7 (9)
Minimum country value***	0 (27)	0 (39)	9,1 (11)	0 (2)	0 (2)

934 * Carcasses of pigs; ** carcasses of < one year old cattle; *** for poultry, only countries having more than ten
935 isolates are considered for minimum value

936 ## *6.2. Resistance in animal target pathogens*

937 ### Resistance in swine pathogens

938 Aminopenicillin resistance in clinical *E. coli* isolates from pigs is very frequent. For example in 2015,
939 the level of amoxicillin resistance was reported as 55% in France (Anses, 2016), nearly 40%
940 (ampicillin) in UK (UK-VARSS, 2015), and close to 40% in Sweden (Swedres-Svarm, 2016) in *E.coli*.
941 The respective figure for amoxicillin-clavulanic acid resistance in France was 18%, while in the UK it
942 was less than 10%. The information was not available for Sweden. Resistance to 3rd-generation
943 cephalosporins was at low level in these reports, but there is variation in which cephalosporins were
944 tested.

945 A multinational report concerning several European countries (El Garch et al., 2016) and a report from
946 Germany (Prüller et al., 2015) both showed that the aminopenicillin MIC distribution for *B.*
947 *bronchiseptica* isolates was unimodal and ranged from 2 to 128 mcg/ml, with high MIC_{50} and MIC_{90}
948 values. All strains in a German study carried the *bla*BOR-1 gene (Prüller et al., 2015), which is

chromosomally located and codes a narrow-spectrum beta-lactamase that is inhibited by clavulanic acid (Lartigue et al., 2005). Thus MICs for amoxicillin-clavulanic acid were lower (MIC_{50} and MIC_{90}, 2 and 4 mg/L, respectively), but the majority of the isolates had a MIC range of 2 - 8 mg/L (Prüller et al., 2015). It is questionable whether these high drug concentrations are achieved with available products and recommended dosages, and if *B. bronchiseptica* should be considered as intrinsically resistant to aminopenicillins in the light of achievable drug concentrations *in vivo*.

For Pasteurellaceae, aminopenicillin resistance is most frequently reported in *Actinobacillus pleuropneumoniae.* An Italian study reported an increasing trend in beta-lactam resistance for this species from 1994 - 2009 (Vanni et al., 2012). The same study also reported high and variable resistance figures for different beta-lactams: 69% for ampicillin, 83% for amoxicillin and 9% for amoxicillin-clavulanic acid. The reason for the difference between ampicillin and amoxicillin resistance figures in that study is not known. Cefiofur resistance was nearly 8% and cefquinome resistance even 24%. In the UK, aminopenicillin resistance was observed in 9 - 17.6% of *A. pleuropneumoniae* isolates, depending on animal population. None was resistant to amoxicillin-clavulanic acid (UK-VARSS, 2015). In France, only 2% were resistant to aminopenicillins, but none to amoxicillin-clavulanic acid or ceftiofur (Anses, 2016). *Pasteurella multocida* is generally susceptible to aminopenicillins (El Garch et al., 2016; (Anses, 2016) or resistance rate is low (UK-VARSS, 2015).

There are several reports indicating that the occurrence of penicillin (and thus aminopenicilin) resistance in *Streptococcus suis* is very low or non-existent in Denmark, Germany, France, The Netherlands and UK (Anses, 2016; Hendriksen et al., 2008; UK-VARSS, 2015), whereas in Poland and Portugal the level of penicillin resistance is 8 - 13% (Hendriksen et al., 2008). Globally, ampicillin resistance in *S. suis* ranges from 0.6 to 23% (Varela et al., 2013).

Resistance in cattle pathogens

Aminopenicillin non-susceptibility is high among cattle clinical *E. coli* ranging from 26% to 85% in different EU countries, depending on year and cattle population. Resistance rates for amoxicillin-clavulanic acid are lower than for ampicillin or amoxicillin. Third-generation cephalosporin resistance is still rather infrequent, although figures up to 7% have been reported in France (Anses, 2016; Swedres-Svarm, 2017; UK-VARSS, 2015). In 280 *E. coli* isolates collected from bovine mastitis across the Europe, amoxicillin-clavulanic non-susceptibility was rare (2.5%) and resistant to 3rd-generation cephalosporins was not observed (Thomas et al., 2015). In a more recent report 7.2% and 1% of *E.coli* from acute mastitis cases had reduced susceptibility to amoxicillin clavulanic-acid and 3rd-generation cephalosporins, respectively (de Jong et al., 2018).

According to a recent report that presented data from ten European countries, cattle respiratory pathogens (*P. multocida*, *M. haemolytica*, *H. somni*) are in general susceptible to aminopenicillins since MICs for amoxicillin were less than 1 mg/L for majority of isolates. Only in *M. haemolytica* was there a bimodal distribution for amoxicillin: 13% (20/149) of *M. haemolytica* isolates had amoxicillin MICs ≥ 32 mg/L. This was not observed for amoxicillin-clavulanic acid which indicates the presence of inhibitor sensitive beta-lactamases in isolates with high amoxicillin MICs (El Garch et al., 2016). Resistance rates are higher in animals in intensively reared conditions, as is the case with veal calves (Catry et al., 2005). Third-generation cephalosporin resistance in cattle respiratory pathogens is rare (Anses, 2016).

In reports contributing eight European countries, penicillin resistance in *S. aureus* from bovine mastitis range from 25 to 36% (de Jong et al., 2018; Thomas et al., 2015) while methicillin resistance rates vary between 0 – 6% (GERMAP, 2016; Gindonis et al., 2013; Swedres-Svarm, 2016). It should be noted though that estimates of methicillin resistance in some reports are based on resistance to certain

penicillinase stable beta-lactams instead of *mec* gene confirmation. The proportion of isolates with reduced susceptibility to penicillin among *Streptococcus uberis* was nearly 30% while none had elevated MICs for amoxicillin clavulanic-acid suggesting that there is no complete cross-resistance between penicillin and aminopenicillins (de Jong et al., 2018).

Resistance in poultry pathogens

According to French and UK surveillance, aminopenicillin resistance in *E. coli* from poultry infections is very common, up to 50% depending on animal age or species in question. Approximately 10% resistance was reported to amoxicillin-clavulanic in *E. coli* , but only a few percent resistance for 3rd-cephalosporins (Anses, 2016; UK-VARSS, 2015). Penicillin/aminopenicillin resistance in *Staphylococcus aureus* from poultry is 0 – 13%, being highest for *S. aureus* isolates in turkeys in France (Anses, 2016).

Resistance in canine and feline pathogens

Antimicrobial consumption or resistance surveillance of companion animal bacteria is performed in some national surveillance programs, such as, the BfT-GermVet in Germany, the Swedish Veterinary Antimicrobial resistance Monitoring [SVARM], and Resapath in France, but EU wide surveillance is lacking. ComPath is a pan-European voluntary program collecting bacterial pathogens from respiratory, dermatological and urinary tract infections of companion animals sponsored by the pharmaceutical industry (De Jong et al., 2013). A collection of a set of bacteria associated with respiratory disease in 2008 - 2010 revealed that canine and feline streptococci and *Pasteurella* spp. isolates were very susceptible to aminopenicillins while the majority of canine *B. bronchiseptica* isolates had amoxicillin-clavulanic acid MIC between 2 - 8 mg/L. Without an inhibitor, amoxicillin MICs for *B. brochiseptica* were ≥ 8 mg/L. Six percent of *Staphylococcus intermedius* group (SIG) isolates were oxacillin-resistant while the majority of *E. coli* were non-susceptible to ampicillin and MICs for amoxicillin-clavulanic acid ranged between 4 and 32 mg/L (Morrissey et al., 2016). Although the number of investigated bacteria in the dataset was small, high amoxicillin-clavulanic acid MICs question the clinical relevance of this drug combination in the treatment of respiratory infections caused by *B. bronchiseptica* or *E. coli* in relation to achievable drug concentrations with approved dosages and drug formulations.

Another ComPath study investigated a set of bacteria from dermatological conditions from dogs and cats in 2008 - 2010. Penicillin resistance in *S. pseudintermedius* was nearly 21% while in *S. aureus* the respective figure was 51%. Comparatively, in the GE*RM*-Vet project, *S. pseudintermedius* isolates (n=54) were collected from dogs with infections of the skin and mucous membranes in 2011 (GERM-Vet, 2015). High resistance rates (~70-75%) to penicillin and ampicillin were found in this survey as well as about 20% resistance to amoxicillin-clavulanate, chloramphenicol, enrofloxacin and gentamicin. In Sweden 3% of *S. pseudintermedius* were MRSP (Swedres-Svarm, 2013), while Schwarz et al. (2007) reported MRSP occurrence ~1% among *S. pseudintermedius* in the BfT-GermVet programme. In the ComPath study, approximately 6% of *S. pseudintermedius* and 5% of *S. aureus* isolates carried a *mecA* gene (Ludwig et al., 2016).

In an European multicenter study on antimicrobial resistance in bacteria isolated from companion animal urinary tract infections (2008-2013), southern countries generally presented higher levels of antimicrobial resistance compared to northern countries (Marques et al., 2016). A temporal increase in resistance to amoxicillin-clavulanate was observed among *E. coli* isolates from the Netherlands and Switzerland, respectively. Multidrug-resistant (MDR) *E. coli* were found to be more prevalent in southern countries (Marques et al., 2016). Regarding other studies, aminopenicillin resistance in canine and feline *E. coli* is 15 – 50% while amoxicillin-clavulanic acid resistance in canine isolates can

be up to 48%. The proportion of third generation cephalosporin resistance was 0 – 31% depending on country and tested substance in question (Anses, 2016; FINRES-Vet, 2017; Swedres-Svarm, 2017). In the ComPath study, five out of 181 (2.8%) Enterobacteriaceae isolates from skin infections had an ESBL or AmpC phenotype. All *E. coli* were classified as non-susceptible to aminopenicillins and their inhibitor combinations with a non-susceptibility breakpoint of 0.5 mg/L (Ludwig et al., 2016). Companion animals can be carriers of multi-drug resistant bacteria such as ESBL/AmpC, MRSA and VRE (Bogaerts et al., 2015; EMA/CVMP, 2015b; Pomba et al., 2017).

6.3. Resistance in human pathogens

European wide surveillance of antimicrobial resistance in human pathogens is organised by European Antimicrobial Resistance Surveillance Network, Ears-Net. The figures are based on 2015 data and on certain pathogens that had been isolated from invasive infections (blood stream infections or cerebrospinal fluid). The data cover 30 EU/EEA countries, although it should be noted that not all countries report resistance figures for each pathogen. In addition the 2015 report provides trend analyses for data collected in 2012 - 2015 (ECDC, 2017a). The resistance figures presented in this section are based on that report unless stated otherwise.

E. coli

There is a high level of resistance in *E. coli* isolates to the aminopenicillins in the EU/EEA and resistance has been stable for years. The EU/EEA population-weighted mean for aminopenicillin resistance was 57% in 2015 ranging from 34% (Sweden) to 73% (Romania), and although no overall trend was detected, increasing trends were observed in seven countries and decreasing trends in four countries. Resistance in *E. coli* to third-generation cephalosporins demonstrated an increasing trend for the EU/EEA population-weighted mean, from 11.9% in 2012 to 13.1% in 2015; and this type of resistance ranged from 1.5% in Iceland to 38.5% in Bulgaria. Significantly increasing trends were observed in 12 countries and decreasing trends in two countries. Of the third-generation cephalosporin-resistant *E. coli*, nearly 87% were confirmed as ESBL-producers, but these data were not available from all countries. Countries also have differences in the definition of ESBLs. Carbapenem resistance was extremely rare in invasive *E. coli* since the EU/EEA population-weighted mean for carbapenem resistance was 0.1% and no trends were observed at the European level. In 23 countries, carbapenem resistance rates were < 0.01% in general, while Greece and Romania reported > 1% carbapenem resistance in *E. coli,* being 1.2% and 1.9%, respectively in these countries. Multi drug resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides in *E. coli* increased from 4.9% to 5.3% in the period of 2012 - 2015.

Salmonella spp.

Although aminopenicillins are not used for treating human salmonellosis, the data from 21 EU MSs and Norway in 2014 indicate that nearly one third of all *Salmonella* spp. isolates from human infections showed resistance to ampicillin (range 11.8-39.8%). Tetracycline and sulphonamide resistance was more or less at the same level and multi-drug resistance phenotype was common (26%). Cefotaxime resistance, was rare (1.1%). Resistance also varies by serovar. (EFSA/ECDC, 2016).

Streptococcus pneumoniae

Susceptibility of *Streptococcus pneumoniae* to penicillin varies between European countries. The percentage of penicillin-non-susceptible isolates (intermediate and resistant) ranged from 0.6% (Belgium) to 39% (Romania). Two countries (Portugal, UK) reported significant increase and two

countries (Belgium, Finland) significant decrease in penicillin non-susceptibility for the period of 2012 - 2015. As the report states, there are variations in methods and interpretation of breakpoints between the countries, population-weighted EU/EEA mean percentage was not calculated for this pathogen. According to the Surveillance Atlas of Infectious Diseases of the ECDC (http://atlas.ecdc.europa.eu/public/index.aspx), in 2015 the proportion of invasive pneumococci showing full resistance to penicillin ranged from 0% (Czech Republic, Luxembourg, Malta, Slovenia) to 26.8% (Romania). Respiratory infections caused by isolates having reduced penicillin susceptibility (intermediate) are generally treatable with high doses of benzylpenicillins or aminopenicillins, while this is not true for meningitis. Although penicillin resistant pneumococci do not show complete cross-resistance to aminopenicillins, their susceptibility to aminopenicillins (or other beta-lactams) can be reduced compared to wild-type isolates. Therefore, if reduced susceptibility to oxacillin (surrogate for penicillin susceptibility) is observed, susceptibility to different beta-lactams should be tested separately. Consequently, it is not possible to estimate the occurrence of aminopenicillin non-susceptibility or resistance based on data published in EARS-Net.

Enterococcus spp.

In general, aminopenicillin non-susceptibility in *E. faecalis* is rare. In 2015, 15 European countries reported less than 1% ampicillin non-susceptibility for this species while nine countries reported 1-10% non-susceptibility for ampicillin. The exceptions were Estonia, Romania and UK with 12.9%, 20.7% and 35.1% non-susceptibility percentages, respectively. In contrast in *E. faecium* aminopenicillin non-susceptibility is very frequent ranging from 67.6% (Romania) to 99.6% (Hungary) in 2015. In 27 countries the occurrence of aminopenicillin non-susceptibility was more than 80% and only in two countries was it less than that (http://atlas.ecdc.europa.eu/public/index.aspx).

S. aureus

There is wide inter-country variation in the occurrence of methicillin-resistant *S. aureus* in Europe ranging from 0% (Iceland) to 57.2% (Romania) while the EU/EEA population-weighted mean was 16.8% in 2015. A two percentage unit decrease in the proportion of MRSA among *S. aureus* at the European level was observed for the period of 2012 - 2015. The surveillance does not cover staphylococcal beta-lactamase-mediated resistance, probably because of its widespread occurrence.

The Ears-Net data do not report or differentiate MRSA on the basis of its origin (i.e. hospital-associated, HA; community-associated, CA; or livestock-associated, LA). Thus surveillance of LA-MRSA is mainly event-based. An EU wide questionnaire survey reported that 22 out of 28 national reference laboratories performed typing of MRSA isolates, but attempts to isolate livestock-adapted CC398 clades from human-adapted clades (such as by the presence of Panton-Valentine Leucocidin genes, PVL) is performed only in few countries. The proportion of LA-associated MRSA isolates (mainly CC398) of all typed MRSA isolates was 3.9% (535/13756). For those countries that reported data only for clinical MRSA isolates (excluding screening isolates; n=9), the corresponding proportion was 9% (417/4612). There is variation between the countries on the proportion of LA-MRSA as well as availability of typing methods. LA-MRSA is less likely to be associated with blood stream infections than other MRSA isolates (Kinross et al., 2017).

7. Possible links between the use of aminopenicillins and their inhibitor combinations in animals and resistance in bacteria of animal origin

In a study with veal calves, the use of penicillins (incl. aminopenicillins) was associated with tetracycline and ciprofloxacin resistance, but not with amoxicillin resistance, in commensal *E. coli* at the farm level. The complexity of the topic was reflected by the result that quinolone and cephalosporin administration had negative association with amoxicillin resistance in that study, while sulphonamide-trimethoprim administration had positive association (Bosman et al., 2014). In a Belgian study ampicillin resistance in porcine *E. coli* was associated with the use of amoxicillin, ceftiofur or enrofloxacin. Regardless of the mode of administration (intramuscular route or oral route under fed or fasting conditions), a 7-day course of ampicillin increased the proportion of the ampicillin resistant faecal *E. coli* population with simultaneous increase of TEM coding beta-lactamase genes (Bibbal et al., 2009). A study that modelled the effect of i.m. ampicillin on *E.coli* in the intestine of pigs indicated that a short treatment duration would result in fewer resistant *E. coli* and return to the pretreatment equibilirium (Ahmad et al., 2016).

In a Belgian study, ampicillin resistance in *E. coli* from piglets and sows was more likely if the animal was exposed to lincomycin-spectinomycin, while ceftiofur resistance was selected by ceftiofur and enrofloxacin use in piglets (Callens et al., 2015). Another study with pigs (Cavaco et al., 2008) revealed that ceftiofur and cefquinome exerted larger selective pressure than amoxicillin, and the effects persisted beyond the withdrawal times for these cephalosporins. Significantly higher counts of cefotaxime resistant coliforms were observed in the three treatment groups than in the control group for up to 22 days after the discontinuation of treatment (Cavaco et al., 2008). The treatment of pigs with amoxicillin or ceftiofur during the rearing period was linked to emergence of cephalosporin-resistant *E. coli*, but these bacteria were no longer present by the time of finishing (Cameron-Veas et al., 2016; Cameron-Veas et al., 2015).

In addition to cephalosporin resistance, amoxicillin and ampicillin are capable of co-selection of multi-drug resistance in *E. coli* (Bibbal et al., 2009; Dheilly et al., 2012; Persoons et al., 2011). In chickens, a two day course of amoxicillin either with a full dose, or 75% of the full dose, selected resistant isolates (van der Horst et al., 2013). This was observed for tetracycline and fluoroquinolones as well, but amoxicillin seemed to have the strongest effect on selection of resistance after a two week follow up-period, although resistance declined in all treatment groups during this period. Aminopenicillins may have a role in maintaining and selecting AmpC- and ESBL-carrying *E. coli* once introduced to a herd, even without use of cephalosporins, as was observed in poultry farms in Denmark (Agersø et al., 2014).

Aminopenicillins are capable of selecting both aminopenicillin resistance and also resistance to other antimicrobials in the gut microbiota of dogs (Edlund and Nord, 2000; Grønvold et al., 2010). In a mouse model, oral *versus* injectable (i.v.) ampicillin significantly resulted in more ampicillin-resistant strains and resistance genes (bla_{CMY-2}) in the gut microbiota (*E. coli*) (Zhang et al., 2013). Of importance, where oral antimicrobial treatments are given to large groups, the resistome in faecal indicator bacteria and pathogens in livestock is much more vulnerable to selection pressure compared to animals kept individually, or in small groups, and if injectable treatment is given (Catry et al., 2016). Therefore interventions to minimize the effect of oral administration of antimicrobials on AMR in the commensal bacteria and target pathogens should be considered. Capability to select resistance may not only depend on the substance, but also on the bacterial species in question since

aminopenicillins have not proven to select for aminopenicillin resistance or resistance to critically important antimicrobials in *Campylobacter* spp. (Elviss et al., 2009; Juntunen et al., 2012).

Possibly due to fact that amoxicillin clavulanic-acid is far less used than aminopenicillins in food-producing animals, there is lack of data on how this combination selects resistance in animals. An *in-vitro* study showed that inhibitor-resistant ESBL-producing *E. coli* CTX-M variants were easily selected under exposure to amoxicillin and clavulanic-acid. The authors presented that this type of selection could also develop *in-vivo* during treatment (Ripoll et al., 2011). Also TEM-1 derived variants with increased resistance to beta-lactam inhibitor combinations were selected by exposing an *E. coli* strain to sub-inhibitory concentrations of amoxicillin and clavulanic-acid (Thomson and Amyes, 1993). Inhibitor resistant bacteria, as well as ESBL and carbapenemase producing bacteria have been observed in food and companion animal species as well as in food of animal origin with increased frequency in this century. In food-producing animals, AmpC/ESBL-mediated resistance is common especially in *E.coli* of poultry origin, possibly due to historical off-label use of ceftiofur in poultry production (Fernández et al., 2018; Madec et al., 2017). Regarding other animals, there are very few studies that investigate the reasons for emergence of ESBL/AmpC/carbapenemases or other multi-drug resistant bacteria. One study demonstrated that healthy dogs with a history of antimicrobial therapy in the previous year had a higher risk of being carriers of ESBL-producing (P=0.003, OR =7.85) and AmpC-producing (P=0.005, OR=6.28) *E. coli* (Belas et al., 2014). During a veterinary hospital outbreak, any antimicrobial use, not just beta-lactam use, was associated with increased likelihood of acquisition of MRSP in dogs and cats (Grönthal et al., 2014). Also quinolone use has been linked to the presence of multi-drug resistant *E. coli* in canine faeces (Leite-Martins et al., 2014). Apart from antimicrobial use, raw food consumption in dogs and cats is associated with higher risk of multi-drug resistant bacteria (Baede et al., 2017; Leonard et al., 2015).

8. Impact of resistance on animal and human health

8.1. Animal health

Aminopenicillins are important for the therapy of a variety of infections in a broad range of animal species. They are categorised as veterinary critically important antibiotics by the OIE. The penicillins class, of which the aminopenicillins made up the largest proportion, was the second most used antimicrobial class in food-producing animals in the EU in 2014 (EMA/ESVAC, 2016). In companion animal species amoxicillin clavulanic-acid is the most used drug of the group. Ampicillin and amoxicillin are the only beta-lactams with extended spectrum authorised for oral administration for food-producing animals.

Aminopenicillin resistance has so far not been described in beta-haemolytic streptococci and the resistance situation in veterinary Pasteurellaceae is good in general, although there is variation in susceptibilities according to the pathogen and animal species in question. For respiratory Pasteurellaceae of cattle and swine, there are alternatives to aminopenicillins, such as tetracyclines, florfenicol, sulphonamide-trimethoprim, or fluoroquinolones, provided that the pathogen is susceptible to one of these agents. If loss of efficacy is due to narrow spectrum beta-lactamase production, amoxicillin-clavulanic acid might be one choice of alternative, noting that the combination is not available, or necessarily suitable for use as, a formulation for oral group treatments.

Loss of efficacy of the aminopenicillins due to acquired resistance has limited their usefulness to treat infections caused by bacterial species belonging to Enterobacteriaceae, which has led to use of amoxicillin-clavulanic acid (although less in food animal species) or other drug classes (e.g.

sulphonamide-trimethoprim, colistin, or fluoroquinolones) to treat infections caused by these species. However, the efficacy of amoxicillin-clavulanic acid compounds for treating systemic infections caused by *E. coli* or other Enterobacteriaceae in food-producing animals is questionable in relation to achievable drug concentrations *in vivo* with recommended dosage schemes. The same applies to respiratory infections caused by *B. bronchiseptica* for which there are better alternatives. In contrast, amoxicillin-clavulanic acid is useful in treating urinary infections or infections where achievable drug concentrations are high due to its pharmacokinetic profile, such as enteric infections. Regarding companion animals, the amoxicillin-clavulanic acid combination may be useful in the treatment of systemic infections caused by Enterobacteriaceae provided that high doses are administered frequently. Non-availability of veterinary authorised substances for intra-venous use restricts the use of amoxicillin-clavulanic acid by this route in companion animals, and side-effects associated with intravenous dosing of human authorized product hamper the usefulness of the combination. Amoxicillin-clavulanic acid is a vital choice in companion animal medicine for treating urinary infections, various skin- and soft tissue infections, such as pyoderma, bite wound infections or infections in which mixed aerobic and anaerobic bacteria are present (Greene, 2013). In general there are not many alternatives available for treating severe infections caused Enterobacteriaceae in companion animals. The options include sulphonamide-trimethoprim combinations, fluoroquinolones and 3rd-generation cephalosporins, although emerging resistance limits the usefulness of the two first mentioned drugs. An available injectable 3rd-generation cephalosporin produces drug concentrations that may not be optimal for treating severe systemic infections.

For treatment of infections due to beta-lactamase-producing staphylococci, cephalosporins or amoxicillin-clavulanic acid are possible options in companion animals. In other animal species these might not be the best choices, or options are limited due to the lack of products authorised for staphylococcal infections. In cattle, anti-staphylococcal penicillins are administered locally to treat mastitis in case the causative isolate is beta-lactamase positive staphylococcus. The loss of efficacy of penicillin or aminopenicillin for equine pathogens would be disastrous due to the very few alternatives in this species.

8.2. Human health

Emerging antimicrobial resistance has consequences both in hospitals and in the community. AMR in general is associated with worse outcomes, including increased rates of complications, additional expenses, higher mortality rates and prolonged hospital stays. Aminopenicillins and their inhibitor combinations are categorised as critically important antimicrobials for human medicine by the WHO, as they are one of the limited therapeutic options for infections caused by *Listeria monocytogenes* and *Enterococcus* spp. and due to the possibility of transmission of *Enterococcus* spp. and Enterobacteriaceae, including *Salmonella* spp. and *E. coli*, from non-human sources to humans. They are among the most commonly used antimicrobials in the EU. In humans, aminopenicillins – with or without beta-lactamase inhibitors - are widely used for the treatment of various community acquired respiratory tract infections, pharyngitis, skin and soft tissue infection as well as urinary tract infections. They are very important drugs for the treatment of infections caused by streptococci, enterococci, *Haemophilus* spp. and *Branhamella* spp.

Beta-haemolytic streptococci are still ubiquitously susceptible to penicillins and aminopenicillin resistance in *Listeria monocytogenes* is very rare. Reduced susceptibility to penicillin in pneumococcus does not necessarily mean reduced susceptibility to aminopenicillins (or other beta-lactams), hence susceptibility to other beta-lactams needs to be tested separately if this is indicated by resistance

screening. In addition, respiratory infections caused by strains with reduced beta-lactam susceptibility are usually treatable by beta-lactams provided increased dosages are used. Other drugs that can be used for pneumococcal infections include, for example, macrolides, tetracyclines or sulphonamide-trimethoprim, but acquired resistance to these drugs is common. The introduction of conjugate vaccines in national vaccination programs by many countries has markedly reduced the number of invasive pneumococcal infections and hospitalisations due to this indication (Principi et al., 2018).

Regarding *Haemophilus influenzae* and *Branhamella catarrhalis,* aminopenicillin resistance is often due to beta-lactamase production and amoxicillin-clavulanic acid is still active against these. If aminopenicillin resistance in *Haemophilus influenzae* is due to changes in penicillin binding proteins, other beta-lactams can still be effective, but their susceptibility should be separately determined. Aminopenicillins alone are not choice for treating human staphylococcal infections due to frequent beta-lactamase production although aminopenicillin with an inhibitor retains activity against beta-lactamase-positive staphylococci. The combination is not active against staphylococci with *mec*-gene mediated resistance (methicillin resistant staphylococci). For treating infections caused by MRSA or other methicillin-resistant staphylococci, available options depend on the susceptibility of the strain in question.

As reviewed in chapter 6, the occurrence of resistance to aminopenicillins in bacteria of human origin is often nearly at the same level (*E. coli*) or exceeds those of veterinary bacterial isolates (*E. faecium*). Lately it has been discussed that the amoxicillin clavulanic-acid combination could be an alternative for the treatment of infections caused by ESBL-strains provided that the isolate is still susceptible to the combination. The combination is not active against AmpC or carbapenemase producing bacteria.

9. Transmission of resistance or resistance determinants between animals and humans

9.1. Transmission of resistant bacteria

Beta-lactamase mediated narrow-spectrum aminopenicillin resistance is very common and extensively distributed in several commensal bacterial species of human and animal origin, and therefore the route and direction of resistance transfer between animals and humans can be very challenging to investigate. Nevertheless, there are several examples demonstrating that drug-resistant bacteria can be transmitted between animals and humans. The flow of transmission is clearly from animals to man in the case of major zoonotic pathogens with a well known food-producing animal reservoir like *Salmonella* spp. and *Campylobacter* spp. , but in many other cases the direction of transmission may be difficult to prove (ECDC/EFSA/EMA, 2015; EMA/EFSA, 2017). Nevertheless, the emergence of multi-drug resistant organisms in food-animal populations has raised concerns that livestock are a source of these bacteria or their resistance determinants for humans.

There is direct and indirect evidence of animal to human transmission of livestock associated MRSA CC398, human to animal transmission of human associated MRSA strains (EMEA/CVMP/SAGAM, 2009). Molecular typing of MRSA isolates has yielded that some lineages are host specific while others are able to colonise or infect a wide variety of animals and humans. The most remarkable livestock associated clone is ST398, which was initially found among pigs, and subsequently was detected in several companion and food-producing animals as well as in humans (Aires-de-Sousa, 2017). MRSA can be transmitted between pet animals and humans, horses and humans, and livestock and humans and the risk for MRSA carriage is higher in humans professionally exposed to animals (Aires-de-Sousa, 2017). Short-term exposure to airborne MRSA poses a substantial risk for farm visitors to become

nasal carriers, but the carriage is typically cleared within hours to a few days. The risk for short-term visitors to cause secondary transmissions of MRSA is most likely negligible (Angen et al., 2017). Food of animal origin is often contaminated with livestock associated MRSA, but also by community associated and hospital associated MRSA strains. Despite this, to date, there is no evidence that consumption of food is associated with increased risk of MRSA colonisation or infection in humans (Aires-de-Sousa, 2017; Larsen et al., 2016). MRSA and MRSP can also be transferred between companion animals and humans (Chanchaithong et al., 2014; Paul et al., 2011; Rodrigues et al., 2017; van Duijkeren et al., 2011; Zomer et al., 2017). *S. pseudintermedius*, including MRSP, can cause infections in humans (Lozano et al., 2017; Somayaji et al., 2016). A MRSP strain has caused a cluster of infections in humans in a tertiary hospital in Sweden (Starlander et al., 2014).

There is direct and indirect evidence that humans and animals share identical ESBLs/AmpC/carbapenemase-producing Enterobacteriaceae, suggesting interspecies transfer (EFSA BIOHAZ Panel, 2011; Hammerum et al., 2014; Marques et al., 2017; Pomba et al., 2017). Some human clinical urinary tract *E. coli* isolates belonging to sequence type (ST) 38 had very few (<15) single nucleotide polymorphism differences when compared with ST38 isolates from poultry meat (Berg et al., 2017). It has been estimated that even 11% of *E. coli* bacteraemia episodes could be of poultry origin (Lazarus et al., 2014), but this was later questioned (Bonten and Mevius, 2015). In general, there is evidence for higher risk for carrying ESBL/AmpC bacteria (Huijbers et al., 2014) or closer similarity of resistance genes or plasmids between human and animal *E. coli*, if humans have close contact with pigs or poultry harbouring ESBL/AmpC *E. coli* (Bonten and Mevius, 2015; Hammerum et al., 2014; Huijbers et al., 2014). However, living in close proximity to livestock animals or farms was not detected to be a risk factor for the transmission of ESBL/AmpC-producing Enterobacteriaceae in humans (Wielders et al., 2017). Another study failed to demonstrate a close epidemiological linkage of ESBL/AmpC genes and plasmid replicon types between livestock farms and people in the general population (Dorado-García et al., 2017). In a Belgian study, the exposure of the consumer to 3rd-generation cephalosporin-resistant *E. coli* (CREC) was modelled, taking into account variables related to the primary production, slaughter, processing and distribution to storage, preparation and consumption of broiler meat. The available baseline data estimated that the probability of exposure to at least 1000 colony forming units of CREC during consumption of a chicken meat was *ca.* 1.5%, the majority of exposure being caused by cross-contamination in the kitchen (Depoorter et al., 2012).

L. monocytogenes is widely distributed in the environment and environmental sources act as reservoirs for human and animal infections. In veterinary medicine, listeriosis in an important disease in ruminant species. Although zoonotic transmission of *L. monocytogenes* is possible either via unpasteurized milk products, meat or *via* direct contact between animals and humans (Godshall et al., 2013), it has been estimated that up to 99% of human listeriosis cases are due to ingestion of food contaminated in the processing factory (EFSA, 2018). Initial contamination may occur at any stage before consumption and the risk of infection can be reduced with careful industrial food processing (e.g. pasteurisation, production hygiene) or, in case of vulnerable individuals, by avoiding food items that may contain listeria (Walland et al., 2015). As addressed earlier in this document, aminopenicillin resistance in *Listeria monocytogenes* is very rare.

E. faecalis of animal origin may be a human hazard as the same lineages can be detected in animals, meat, and humans in the community and hospitals, while *E. faecium* isolates from human clinical outbreaks are usually different to *E. faecium* from animals, food, and humans in the community, indicating that they do not constitute direct human hazard, although they could act as donors of antimicrobial resistance genes for other enterococci (Hammerum, 2012).

JIACRA II report investigated ecological associations between the consumption of certain antimicrobial agents and occurrence of resistance in bacteria from food-producing animals and humans (ECDC/EFSA/EMA, 2017). The report confirmed the positive association between antimicrobial use and resistance in both humans and food-producing animals highlighting the need for prudent use and to reduce the antimicrobial consumption in both sectors. The report also indicated that associations between the antimicrobial consumption in food-producing animals and resistance in human pathogens are not straightforward. For example, positive associations between the fluoroquinolone consumption in food-animals and fluoroquinolone resistance in *Salmonella* spp. and *C. jejuni* from humans were detected, while resistance to 3rd-generation cephalosporins in human *E.coli* was only associated with consumption of 3rd- and 4th-generation cephalosporins in humans (ECDC/EFSA/EMA, 2017). Although the report did not investigate the consumption of aminopenicillins in food-animal species and aminopenicillin or other resistance in human bacteria, the results show that the epidemiology of resistance is complex and several factors other than the amount of antimicrobials consumed may influence the level of resistance.

9.2. Transmission of resistance determinants

The gene *bla*CMY-2 confers resistance to aminopenicillins, extended-spectrum cephalosporins and the inhibitor clavulanate. In a Norwegian study, *E. coli* resistant to extended-spectrum cephalosporins recovered from retail chicken meat and carrying an IncK plasmid with the *bla*CMY-2 gene (N=17) were compared by whole genome sequencing with human clinical *E. coli* isolates (N=29) which also carried an IncK plasmid bearing the *bla*CMY-2 gene. The plasmid in all 29 human *E. coli* isolates was highly similar to that present in the poultry isolates (Berg et al., 2017). *E. coli* ST38 with *bla*CMY-2 has been detected in broilers in different Nordic countries suggesting clonal expansion of this strain in broilers (Myrenås et al., 2018). The main beta-lactamase enzymes conferring resistance to aminopenicillins (and in some cases aminopenicillin inhibitor combinations) are shown in

Table **1**. The TEM-1 beta-lactamase is encoded by the *bla*$_{TEM-1}$ gene which is carried by two of the first transposons to be identified; Tn*3* which carries *bla*$_{TEM-1a}$ and Tn*2* which carries *bla*$_{TEM-1b}$. The genes *bla*$_{TEM-1a}$ and *bla*$_{TEM-1b}$ differ slightly but encode the same enzyme. The enzyme TEM-2 differs from TEM-1 by only a single amino acid change and is carried by Tn*1*. All TEM variants are thought to have originated by mutation from TEM-1, whilst Tn*1*, Tn*2* and Tn*3* are all related by homologous recombination (Partridge and Hall, 2005). Carriage of *bla*$_{TEM}$ by mobile genetic elements probably accounts for its extremely widespread, near ubiquitous occurrence. A further mobile genetic element (IS26) is present in the related *Tn*A transposons and the location of IS*26* and the *bla*$_{TEM}$ gene has been of use in demonstrating epidemiological links. For example the same variant of Tn*6029* was detected in *Salmonella* Typhi from Vietnam in 1993 on an IncHI1 plasmid and also on a commensal human *E. coli* from Australia in 2008 where it was no longer present on an IncHI1 plasmid, confirming spread between bacterial species, geographically and between different genetic locations (Bailey et al., 2011).

SHV beta-lactamases are thought to have all evolved from the chromosomal beta-lactamase of *K. pneumoniae* and occur primarily on plasmids. These beta-lactamases are also frequently associated with the IS*26* element and it has been suggested that this element may transpose preferentially in plasmids (Liakopoulos et al., 2016). Carriage of SHV beta-lactamases by a number of different plasmid types has facilitated widespread dissemination into diverse ecological niches including surface waters, food-producing animals and food such as retail meat derived from animals (Liakopoulos et al., 2016). TEM and SHV beta-lactamases have not been detected in integrons because they do not appear to be able to form suitable gene cassettes (Poirel et al., 2008).

Salmonella Genomic Island 1 (SGI1) is an integrative mobile genetic element carrying integrons with multiple antimicrobial resistance genes which was first detected in *S.* Typhimurium definitive phage type (DT)104 (Boyd et al. 2001). SGI1 and variants of SGI1 have subsequently been detected in a range of salmonella serovars as well as in *P. mirabilis* (Mulvey et al., 2006; Ahmed et al., 2007). SGI1 and its variants have been detected in *S. enterica* serovars Agona, Albany, Derby, Kentucky, Newport and Java (Paratyphi B dT+) (Beutlich et al., 2011). The antimicrobial resistance genes carried by SGI1 classically include the genes conferring pentavalent resistance in *S.* Typhimurium DT 104 to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines. The gene conferring resistance to ampicillin is *bla*$_{CARB-2}$(also referred to as *bla*$_{PSE-1}$). Integrons are genetic elements, possessing a site-specific recombination system, which are able to capture and express gene cassettes; these gene cassettes frequently include genes conferring antimicrobial resistance. Integrons commonly occur on plasmids or transposons and play a major role in the acquisition of resistance genes by Gram-negative bacteria (Leverstein-van Hall et al., 2003). Class 1 integrons often also possess the sulphonamide resistance gene *sul1* downstream of the gene cassette (Leverstein-van Hall et al., 2003) and this accounts for the frequent occurrence of sulphonamide resistance as a component of multi-drug resistance patterns (which also often include resistance to aminoglycosides e.g. streptomycin) (Leverstein-van Hall et al., 2003). The first report of *bla*$_{CARB-2}$ carried on an integron was from a plasmid in *P. aeruginosa*, and although integrons with *bla*$_{CARB-2}$ have also been detected in other organisms, including *K. pneumoniae*, *P. mirabilis* and *A. baumanii*, it has been considered to occur mainly in *S. enterica* (Domingues et al., 2015). The widespread dissemination of *S*. Typhimurium DT104 has also resulted in dissemination of the integron and *bla*$_{CARB-2}$ resistance gene it usually carries. Food-borne zoonotic transmission of *S*. Typhimurium DT104 from animals to man (as well as transmission through direct contact with animals), provides a means of transmission of resistance between animals and man.

The ROB-1 beta-lactamase gene, belonging to class A-beta-lactamases, has been described in *A. pleuropneumoniae* (Juteau et al., 1991) The same gene has been detected in other bacterial species

belonging to the family Pasteurellaceae isolated from animals and humans (Livrelli et al., 1991) and is considered to be of animal pathogen origin (Medeiros et al., 1986). The plasmid encoded beta-lactamase ROB-1, detected in *A. pleuropneumoniae* isolates from pigs, was also detected in the human meningitis pathogen *H. influenzae* Type b in the USA (Medeiros et al., 1986), although the majority of beta-lactam resistance in *H. influenzae* was related to the presence of the beta-lactamase TEM-1, which is extremely widespread in bacteria from both human and animal bacteria. The plasmids carrying ROB-1 were found to be very similar in both *A. pleuropneumoniae* and *H. influenzae* Type b suggesting transfer between these bacterial species. The available epidemiological information did not indicate direct contact with pigs in human cases of meningitis *H. influenzae* Type b carrying ROB-1 (Medeiros et al., 1986). ROB-1 has also been detected in other animal pathogens belonging to the family Pasteurellaceae including *M. haemolytica* and *P. multocida* (Azad et al., 1992, Livrelli et al., 1988), suggesting exchange of ROB-1 plasmids between these species.

Ampicillin resistance can predict the presence of integrons in Enterobacteriaceae (Leverstein-van Hall et al., 2003), although TEM and SHV beta-lactamases are not carried by integrons (Poirel et al., 2008). Linkage through co-location of bla_{TEM} and integrons on plasmids was considered to account for the predictive value of ampicillin resistance regarding the presence of integrons (Leverstein-van Hall et al., 2003). It was also shown that in randomly-selected *E. coli* from man (originating from both European hospitals and community settings), combined resistance to ampicillin (and/ or piperacillin) and sulphonamides (trimethoprim) was the common core resistance pattern of >90% of resistant isolates, showing it was the probable common starting point, from which further resistance was acquired.

Until the 1990s, the main ESBLs identified in human clinical isolates were SHV or TEM ESBL variants, but later CTX-M type enzymes emerged (Argudín et al., 2017). During the last 15 years, ESBL-producing TEM, SHV and CTX-M or AmpC-producing, CMY-carrying Enterobacteriaceae (mainly *E. coli* and *Salmonella* spp.) have also been increasingly reported in food-producing animals and food (EFSA BIOHAZ Panel, 2011). The distribution of different ESBL-enzymes is similar in bacteria of animal and human origin. The different incompatibility group (Inc) plasmids, such as IncN, IncI, IncF and IncK, and IncP have been associated with genes coding CTX-M enzymes (Argudín et al., 2017; Franco et al., 2015). A study that utilised a whole genome sequencing technique resulted that while there were overlaps in antimicrobial resistance genes in bovine and human associated *Salmonella* spp., especially in *Salmonella* Newport, many antimicrobial genes were confined to human isolates (Carroll et al., 2017). A population study conducted in Italy in broiler chicken flocks, broiler meat, and humans demonstrated by whole genome sequencing and bioinformatics analysis that human cases of Salmonellosis by *S.* Infantis were caused by an emerging clone of ESBL (CXTM-1)-producing S. Infantis spreading in the broiler chicken industry since 2011, and that the ESBL gene was carried by a (IncP) conjugative mosaic megaplasmid (Franco et al., 2015). Another study with the same technique revealed that transmission of common CMY-2 plasmid may occur among *S.* Heidelberg strains with variable genetic backgrounds and different animal, environmental or human sources (Edirmanasinghe et al., 2017). On the other hand, ESBL-producing *E. coli* from environmental, human and food specimens in Spain showed high clonal diversity with some clonal complexes observed in all specimens (Ojer-Usoz et al., 2017). A Dutch study showed distinguishable ESBL/AmpC *E. coli* transmission cycles in different hosts and failed to demonstrate a close epidemiological linkage of ESBL/AmpC genes and replicon types between livestock farms and people in the general population (Dorado-García et al., 2017). The mechanisms of spread of CTX-M enzymes are diverse and can involve insertion sequences, transposons, class 1 and other integrons; the diversity of available mechanisms of spread is considered to have enhanced their dissemination (Poirel et al., 2008). Within recent years, also bacteria carrying acquired carbapenemases, such as VIM-1 producing *E. coli* and *Salmonella* spp., OXA-23 and NDM-1

positive *Acinetobacter* spp. have emerged in pigs, cattle and poultry (Guerra et al., 2014). Carbapenemases (NDM-1 in *E. coli*, OXA-48 in *E. coli* and *K. pneumoniae* and OXA-23 in *Acinetobacter* spp.) have been detected also in bacteria of companion animals environmental specimens (Abraham et al., 2014; Woodford et al., 2013). All these have also been detected in bacteria of human origin, and with far higher frequency than in animals, suggesting that their origins are from human sources. Carbapenems are not authorised for animal use in the Europe, but the use of other antimicrobials could co-select carbapenemase-producing bacteria in the animal population following the introduction of such bacteria.

Similarity of SCC*mec*-elements between human and animal MRSA or MRSP strains suggests that this element is transferrable between staphylococci of animal and human origin. Closely related *mecA* allotypes with chromosomal location, but without being part of SCC*mec*, have been described in *Staphylococcus sciuri* group staphylococci that are animal commensals suggesting that origin of the *mecA* could be staphylococci belonging to this group. Also the origin of *mecC* gene have been suggested to be in animal staphylococci (Argudín et al., 2017). On the other hand, *mec*A carrying staphylococci started to emerge in the human population first in hospitals in the 1960's and later in the community in humans, far earlier than in animal population (Aires-de-Sousa, 2017).

10. Discussion

Aminopenicillins including their beta-lactamase inhibitor combinations are very important drugs in veterinary and human medicine. Although aminopenicillins are seldom the sole treatment option (with the exception of therapy for Listeria and enterococci in humans) they are often used as first line antimicrobials for a variety of infections in both animals and humans. In food-producing animals ampicillin and amoxicillin make up the major proportion of penicillins used while in companion animals the amoxicillin clavulanic-acid combination is favoured. However, there are significant differences between countries. The fact that benzyl penicillin and its pro-drugs are favoured over aminopenicillins in Nordic countries whilst the opposite is true in central and southern Europe suggests that there are differences in the manner and habits of antimicrobial usage (e.g. whether group medication is favoured instead of individual animal treatment). However, differences in sales volumes can also be due to differences in availability of beta-lactams (e.g. lack of availability of a narrow-spectrum beta-lactam formulation for oral use in food-producing animals), production structures (e.g. dominant food-producing animal species), herd sizes, disease occurrence and production facilities.

Extensive use of aminopenicillins (incl. their inihbitor combinations) in both human and veterinary medicine has led to the selection and spread of aminopenicillin resistance, with a range of different genetic bases. Although the major selection force for extended spectrum cephalosporin resistance is considered to be the use of cephalosporins and fluoroquinolones, aminopenicillins, especially inhibitor combinations, may co-select such resistance as can several other antimicrobials if the organism harbours the determinants conferring resistance to cephalosporins and fluoroquinolones in addition to aminopenicillin resistance. The same or similar resistance genes have been isolated in bacteria of human and animal origin, and molecular studies suggest that resistance gene transmission or transmission of bacteria with resistance to aminopenicillins occurs between bacteria of animal, human, food or environmental origin (Madec et al., 2017). Due to the complexity of AMR epidemiology and the near ubiquity of some aminopenicillin resistance determinants, the direction of transfer – whether gene or resistant isolate - may be difficult, if not impossible, to ascertain, except for major food-borne zoonotic pathogens like *Salmonella* spp., and certain LA-MRSA clones. Recent evidence suggests the

highest similarities (in ESBL/AmpC procuding *E. coli)* among livestock and their respective farming communities but not with the general population at large (Dorado-García et al., 2017). Nevertheless, the existence of these common resistance determinants in animal bacteria has raised concern about food-producing animal reservoirs for antimicrobial resistance (EFSA BIOHAZ Panel, 2011), which is of major concern for zoonotic pathogens causing illness in humans (*Salmonella* and *Campylobacter* spp., and LA-MRSA). In all, the epidemiology of resistance is complex and factors other than the amount of antimicrobials consumed may influence the level of resistance.

Studies into the capability of different antimicrobials to select resistance are numerous, but results vary between them, as do the methodologies used. Aminopenicillins without a beta-lactamase inhibitor probably select narrow-spectrum beta-lactamases while their inhibitor combinations also select inhibitor resistance. However, aminopenicillins without inhibitors are also able to co-select extended spectrum beta-lactam or multi-drug resistance due to simultaneous carriage of several resistance genes by many bacterial isolates. Apart from antimicrobial use, the extent of resistance selection and its dissemination is affected by many other factors, such as animal density and the route of administration. Currently there is no evidence indicating that the use of aminopenicillins in animals would be associated with aminopenicillin or other resistance in human bacteria. More research is needed to explore AMC in food-producing animals and AMR in humans.

It is clear that resistant organisms are transferred between animals and humans, but the direction and magnitude of transfer is often difficult to prove or quantify, except for the major food-borne zoonotic pathogens. Resistance can be spread vertically as an emergence of resistant clones or horizontally *via* plasmids or other transmissible gene elements. The risk of resistance transfer may depend on several factors related to the host animal and complicated bacterial inter-relationships. Also the length and closeness of contact and route of transfer (via skin contact or contaminated food) may affect the magnitude of the risk of resistance transfer from animals to humans and vice versa. There is evidence that humans who have contact with livestock have a higher chance of carrying multi-drug resistant bacteria, such as ESBL-producing *E. coli* or LA-MRSA, compared to humans with no animal contact, whilst the risk for resistance transfer by consumption of food of animal origin is considered low, especially if good food hygiene practices are followed.

Considering that resistance to aminopenicillins (without inhibitors) is at a very high level in some organisms (as is the case with *E. coli*), that these substances have been extensively used both in veterinary and human medicine for decades, it may be difficult to estimate to what extent the use of aminopenicillins in animals, could create negative health consequences to humans at the population level. Despite these challenges, there have been some attempts to model the effects of veterinary antimicrobial consumption on human health. Risk estimates range from a few additional illnesses per million at risk to thousands, depending on the antimicrobial substance and pathogen in question. For example, the public health risk from ampicillin-resistant *E. faecium* due to veterinary use of penicillins in food-producing animals was estimated to be very low or non-existent (McEwen, 2012). JIACRA II (ECDC/EFSA/EMA, 2017) pointed out associations between fluoroquinolone consumption in food-animals and fluoroquinolone resistance in zoonotic bacteria of humans while such association was not detected for 3rd and 4th-generation cephalosporins. While this report did not estimate the association of aminopenicillin consumption and antimicrobial resistance, it confirmed the positive association between AMC and AMR in both humans and food-producing animals highlighting the need for prudent use and to reduce the AMC in both sectors.

Although the direct risk of veterinary antimicrobial use to humans would be lower compared to the risk from their use in human medicine, it is evident that veterinary use of antimicrobials increases the

selection pressure towards AMR in animals and the environment and jeopardises at least animal health and welfare. Aminopenicillin use in animals may select resistance in zoonotic or other bacteria of animal origin that can further be transferred to humans, but based on the extent of use of these drugs in humans, the major resistance selection pressure in human pathogens caused by aminopenicillin use in European countries can be considered to be due to human consumption of these or other related beta-lactam drugs.

Based on an assessment of current use and resistance profiling, it may be possible to make recommendations to limit the further development of resistance to both aminopenicillins and other important related classes of antimicrobials. Antimicrobial use in general should be reduced in veterinary medicine to safeguard future animal health and welfare and to reduce unnecessary selective pressure for antimicrobial resistance in the ecosystem. Tools include improvements in hygiene in between livestock production cycles and animal husbandry at large, vaccinations, proper diagnostics and avoidance of use of antimicrobials prophylactically to animals having no signs of infection. Also, the route of administration should be considered to reduce selection pressure in the gut microbiota. Mass medication of food-producing animal flocks by oral route facilitates the selection and spread of resistance and attempts to reduce such use are needed. Current indications should be reviewed in relation to authorised dosing schemes in order to ensure achievement of sufficient PK/PD targets and subsequently minimising the risk for resistance selection. This is especially true for inherently less susceptible organisms such as Enterobacteriaceae and *Bordetella bronchiseptica*. Animal species and bacterial/infection specific breakpoints should be established to ensure the proper use of these substances.

11. Conclusions

The AMEG categorisation considers the risk to public health from AMR due to the use of antimicrobials in veterinary medicine. The categorisation is based primarily on the need for the antimicrobial in human medicine, and the risk for spread of resistance from animals to humans. Aminopenicillins are important in human medicine in terms of the high extent of their use to treat a variety of important infections. *Listeria monocytogenes* and *Enterococcus* spp. were identified by WHO as human pathogens for which there are few treatment alternatives to aminopenicillins available. Animals could serve as a reservoir for aminopenicillin resistance in *E. faecalis* and *L. monocytogenes*, but such resistance is very rare. In addition, although aminopenicillins are important as first choice for the treatment of enterococcal infections in humans, there are alternatives of last resort (e.g. vancomycin, linezolid, tigecycline).

Use of aminopenicillins in animals creates a selection pressure for beta-lactam resistance. In common with several other antimicrobial classes, aminopenicillins can select LA-MRSA which can be transferred to humans via contact with livestock. Resistance to aminopenicillins is very frequent in Enterobacteriaceae, including *Salmonella* spp., from food-producing animals in the EU. For example, aminopenicillins can select MDR *S*. Typhimurium DT104 which may be transmitted via the foodborne route from animals to man.

Commensal bacteria in animals, such as Enterobacteriaceae, may act as a reservoir for resistant bacteria or resistance genes that may be transferred to bacteria in humans; however, the high extent of aminopenicillin use in humans itself provides a selection pressure for resistance in the human microbiota. The significance to public health of additional aminopenicillin resistance transferred from animals is considered to be low. Although amoxicillin clavulanic acid combinations have very low use in food-producing animals, AmpC/ESBL resistance mechanisms confering resistance to 3rd and 4th

generation cephalosporins, have emerged in Enterobacteriaceae from animals in recent years and the combination has higher potential to select further these types of resistance than aminopenicillins alone.

It should also be considered that aminopenicillins, and to a lesser extent amoxicillin-calvulanic acid combinations, have been widely used for decades in veterinary medicine in the EU, and that they are categorised as veterinary CIAs by the OIE on the grounds that they are very important in the treatment of many diseases in a broad range of animal species.

All these factors should be taken into account for the AMEG's categorisation, which is currently under review. It is suggested that the AMEG could give consideration to a further stratification of the categorisation to allow a distinction in the ranking between those substances currently in Category 2 (fluoroquinolones, 3rd- and 4th-generation cephalosporins and colistin, for which there are fewer alternatives) and the amoxicillin-clavulanate combinations, and between the latter and the straight aminopenicillins. Amoxicillin-clavulanate has a wider spectrum and thus it is likely that it has higher chance to select multidrug resistant organisms compared to aminopenicillin alone.

1598 **Table 5.** The use of aminopenicillins and examples of their indications in veterinary medicine in the EU. Indications are collected SPCs of authorised
1599 veterinary products in UK, France, Spain and Germany.

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Substance	Volume of use (ESVAC, 2015)	Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume	Duration of use	Species	Disease
Amoxicillin	1826 tonnes	Vast majority of sales are for oral use, 4% of sales of amoxicillin VMPs are injectable preparations. Sales of intramammary (0.1%) and intrauterine preparations (0.1%) are very low	Premix is authorised for up to 15 days treatment for pigs	Pigs	Respiratory (incl. *Actinobacillus pleurpopneumoniae*) and gastrointestinal tract infections (incl. salmonellosis), meningitis (*Streptococcus suis*), arthritis
			Drinking water formulations are administered for 3-5 days to pigs and poultry	Chickens and other poultry	Respiratory (incl. *E coli*) and gastrointestinal tract infections
			'Top dressing' on fish feed for 10 days	Atlantic salmon	Furunculosis due to *Aeromonas salmonicida*
			Intramammary preparations administered for 3	Cattle	(Sub)clinical mastitis

Substance	Volume of use (ESVAC, 2015)	Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume	Duration of use	Species	Disease
			milkings		
			Oral bolus for 3 days Tablets for 5-7 days; and longer (e.g. 4 weeks) for chronic infections.	Calves dogs and cats	Enteritis, omphalitis, respiratory, periodontal, gastrointestinal, urogenital and skin/soft tissue infections. A wide range of G+ and G- bacteria are included as named pathogens, including: *Bordetella bronchiseptica*, *E coli*, *Pasteurella* spp., *Proteus* spp., *Staphylococcus* spp. (penicillin-sensitive), and *Streptococcus* spp.
			Injectables are indicated for 3-5 days treatment	Cattle, pigs, sheep, dogs, cats	Respiratory, gastrointestinal and urogenital tract infections, ear, eye and soft tissue infections A wide range of G+ and G- bacteria are included as named pathogens, including: *Actinobacillus* spp., *Bordetella bronchiseptica*, *Clostridium* spp., *Erysipelothrix rhusiopathae*, *E. coli*, *Haemophilus* spp., *Pasteurella* spp., *Moraxella* spp., *Fusiformis* spp, *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Trueperella* spp.

Substance	Volume of use (ESVAC, 2015)	Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume	Duration of use	Species	Disease
Amoxicillin + clavulanate	Contributes 0.8% of total sales of penicilllins in mg/PCU in the EU.		Drinking water formulations are administered for 5 days to pigs	Pigs	Treatment of respiratory infections (*Actinobacillus pleuropneumoniae*, *Pasteurella* spp), meningitis (*Strep. suis*), gastrointestinal infections (*Clostridium perfringens*, *E. coli*, *Salmonella typhimurium*)
			Intramammary preparations	Lactating cattle	Clinical mastitis caused by *Staphylococcus* spp., *Streptococcus* spp., *Trueperella* pyogenes and *E. coli*.
			Tablets and oral drops for 5-7 days; and longer for chronic cases	Dogs and cats	Treatment of infections of skin and soft tissue, urinary tract, respiratory tract, enteritis. A wide range of G+ and G- bacteria are included as named pathogens, including: *Bordetella bronchiseptica*, *E.coli*, *Clostridium* spp., *Pasteurella* spp., *Proteus* spp., *Staphylococcus* spp. and *Streptococcus* spp.

Substance	Volume of use (ESVAC, 2015)	Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume	Duration of use	Species	Disease
			Injectable preparations are indicated for 3-5 days treatment	Cattle, pigs, dogs, cats	Respiratory, gastrointestinal and urogenital tract infections, ear, eye and soft tissue infections A wide range of G+ and G- bacteria are included as named pathogens, including strains resistant to amoxicillin alone, including: *Actinobacillus* spp., *Actinomyces bovis*, *Bacteroides*, *Bordetella bronchiseptica*, *Campylobacter* spp., *Clostridium* spp., *Erysipelothrix rhusiopathae*, *E. coli*, *Haemophilus* spp., *Klebsiella* spp., *Pasteurella* spp., *Moraxella* spp., *Salmonella* spp., *Staphylococci* spp., *Streptococcus* spp., *Trueperella* spp.
Ampicillin	48 tonnes	67% of sales are for oral use, but one-third for injectable formulations. Sales of intramammary and intrauterine presentations add up to approximately 4% of sales of ampicillins.	Drinking water formulations for 3 days	Calves, lambs, foals, poultry	Gastrointestinal infections
			Tablets are authorised for 5 days' treatment	Cats and dogs	Respiratory, gastrointestinal and urinary tract infections including those due to: *Streptococcus* spp., *Pasteurella* spp., *Staphylococcus* spp.
			Injectables are	Cattle,	Respiratory, gastronitestinal and urogenital tract

Substance	Volume of use (ESVAC, 2015)	Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume	Duration of use	Species	Disease
			indicated for up to 5 days treatment	sheep, goats, pigs, dogs, cats	infections, meningitis, septicaemia. A wide range of pathogens including *Bordetella bronchiseptica*, *Erysipelothrix rhusiopathae*, *Mannheima haemolytica*, *Pasteurella* spp., *Staphylococci* spp. (penicillin sensitive), *Streptococcus spp*., *Trueperella* spp.
Ampicillin + cloxacillin	No usage estimates available		Intramammary preparations	Cattle	Clinical mastitis, dry cow therapy. G+ and G- organisms: *Streptocccus* spp., *Staphylococcus* spp. (penicllin sensitive), *E. coli*, *Trueperella pyogenes*

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Appendix

Table A1. List of ATC codes of extended spectrum penicillins and their inhibitor combinations. Veterinary authorised substances have been marked with an asterisk (*)

J01CA Penicillins with extended spectrum		J01CR Combinations of penicillins, incl. beta-lactamase inhibitors	
J01CA01	ampicillin*	J01CR01	ampicillin and enzyme inhibitor
J01CA02	pivampicillin	J01CR02	amoxicillin and enzyme inhibitor*
J01CA03	carbenicillin	J01CR03	ticarcillin and enzyme inhibitor
J01CA04	amoxicillin*	J01CR04	sultamicillin
J01CA05	carindacillin	J01CR05	piperacillin and enzyme inhibitor
J01CA06	bacampicillin	J01CR50	combinations of penicillins
J01CA07	epicillin		
J01CA08	pivmecillinam		
J01CA09	azlocillin		
J01CA10	mezlocillin		
J01CA11	mecillinam		
J01CA12	piperacillin		
J01CA13	ticarcillin		
J01CA14	metampicillin		
J01CA15	talampicillin		
J01CA16	sulbenicillin		
J01CA17	temocillin		
J01CA18	hetacillin		
J01CA19	aspoxicillin		
J01CA20	combinations		
J01CA51	ampicillin, combinations		