EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 July 2017
EMA/CVMP/AWP/721118/2014
Committee for Medicinal Products for Veterinary Use (CVMP)

# Reflection paper on use of aminoglycosides in animals in the European Union: development of resistance and impact on human and animal health

Draft

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| Draft agreed by Antimicrobials Working Party (AWP) | 24 May 2017 |
| Adopted by CVMP for release for consultation | 13 July 2017 |
| Start of public consultation | 25 July 2017 |
| End of consultation (deadline for comments) | 31 October 2017 |

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# Executive summary

Aminoglycosides (AGs) are important antibacterial agents for the treatment of various infections in humans and animals, although they are seldom the sole treatment option. In veterinary medicine in the European Union (EU), AGs account for 3.5% of the total sales of antimicrobials. The most frequently used AGs are neomycin, dihydrostreptomycin and spectinomycin and approximately half of the total use is in oral forms. In human medicine AGs, especially gentamicin, tobramycin and amikacin, are used primarily in infections involving multidrug-resistant Gram-negative bacteria, such as *Pseudomonas*, *Acinetobacter*, and *Enterobacter* and they are mainly applied systemically. Following extensive use of AGs in humans, food-producing animals and companion animals, acquired resistance among human and animal pathogens and commensal bacteria has emerged. Acquired resistance occurs through several mechanisms, but enzymatic inactivation of AGs is the most common one. Resistance mechanisms differ between the AG molecules and between bacterial species. Cross-resistance to several AGs by a single mechanism/plasmid does occur, but generally there is no complete cross resistance to all AGs by one mechanism. Mechanisms conferring resistance to (dihydro)streptomycin and spectinomycin usually differ from those of the other AGs. AG resistance has been found in many different bacterial species, including those with zoonotic potential. Resistance to streptomycin and spectinomycin is generally high in veterinary pathogens, while resistance to gentamicin is still uncommon for most bacteria originating from animals. In *E. coli,* Salmonella and Campylobacter isolates from food-producing animals in EU member states (MS) resistance to gentamicin is scarce, whereas resistance to streptomycin in *E. coli* and in some MS also in Salmonella and Campylobacter isolates is common. In livestock-associated MRSA CC398, resistance to gentamicin is commonly found. There is evidence that the usage of AGs in human and veterinary medicine is associated with the increased prevalence of resistance. Resistance genes are often located on mobile elements facilitating their spread between different bacterial species and between animals and humans. The same resistance genes have been found in isolates from humans and animals. Evaluation of risk factors indicates that the probability of transmission of AG resistance from animals to humans through transfer of zoonotic or commensal food-borne bacteria and/or their mobile genetic elements can be regarded as high. For human medicine, gentamicin, tobramycin and amikacin are of greater importance than the other AGs. Resistance to gentamicin, tobramycin and amikacin is generally still scarce in veterinary organisms and use of these AGs in animals is more often through local administration or by injection. AGs are important in human medicine for the treatment of MDR tuberculosis, Gram-negative infections and enterococcal/streptococcal endocarditis and have been categorized by WHO as critically important for human medicine. AGs are, however, rarely the sole treatment option in either veterinary or human medicine.

Considering the AMEG criteria, veterinary-authorised AGs would be placed in Category 2 given (i) their importance in human medicine and (ii) the high potential for transmission of resistance determinants between animals and humans and the potential for co-selection of resistance as described by the AMEG. However, according to the CVMP, AGs have a lower risk profile compared to fluoroquinolones and 3$^{rd}$- and 4$^{th}$-generation cephalosporins as they are used for a lower absolute number of individuals affected by all diseases for which these antimicrobials are one of few therapies available, and they are used less often for other infections than 3$^{rd}$- and 4$^{th}$-generation cephalosporins and fluoroquinolones in human medicine (WHO). It is suggested that AMEG could give consideration to a further stratification of the categorization.

# CVMP Recommendations for action

In April 2013, the European Commission (EC) requested advice from the European Medicines Agency (EMA) on the impact of the use of antibiotics in animals on public and animal health and measures to manage the possible risk to humans. The advice was provided by the Antimicrobial Advice ad hoc Expert Group (AMEG). As part of the advice, the AMEG provided a categorisation of antimicrobials according to their risk for public health. This CVMP/AWP reflection paper considers a recommendation from the AMEG for further risk profiling to be undertaken for the aminoglycosides (AGs) to enable them to be placed within the AMEG's categorisation.

In veterinary medicine AGs are used to treat a wide range of infections in all major food-producing animals and in companion animal species. In particular, they are important for treatment of post-weaning diarrhoea in pigs, for topical treatment of *Pseudomonas* spp. infections in companion animals and gentamicin is used for treatment of Gram-negative infections in horses. AGs are rarely the only treatment option for specific infections. AGs (in particular (dihydro)streptomycin and neomycin) are also used in combination with other antimicrobials, often beta-lactams, to achieve a synergistic effect or to broaden the spectrum of activity.

In 2014, AGs accounted for 3.5% of the total sales of veterinary antimicrobials in mg/PCU in 29 EU countries (EMA/ESVAC, 2016). The substances with the highest volume of use were neomycin, dihydrostreptomycin and spectinomycin.

AG resistance mechanisms are complex and differ between AG molecules and bacterial species. There is usually no complete cross-resistance between antimicrobials in this class, although there is evidence that use of apramycin in pigs may select for gentamicin-resistant *E. coli*. Amongst animal pathogens, high levels of resistance have been reported to various AGs in isolates of *Streptococcus suis* from pigs, and to streptomycin in *E. coli* from poultry, pigs and equids. In isolates from food-producing animals collected under mandatory EU surveillance of zoonotic and indicator bacteria (EFSA/ECDC, 2017), resistance to streptomycin was generally very common, whereas it was low for other tested AGs, with some variation between MSs and animal species. Resistance to various AGs has also been reported to occur commonly in LA-MRSA isolates from pigs, veal calves and poultry in the Netherlands (de Neeling et al., 2007; Wagenaar and Van de Giessen, 2009; Wendlandt et al., 2013b). Enterobacteriaceae, LA-MRSA and *Enterococci* spp. have potential for zoonotic transmission of genes encoding resistance to AGs and similar resistance genes and mobile elements have been found in bacteria from humans and animals. Based on the AMEG's criteria, the probability of transfer of AG resistance genes from animals to humans is estimated as high (Table 4).

AGs are classified by WHO as critically important antimicrobials (CIAs) in human medicine, although they are not included with the highest priority CIAs. In acute care in human medicine, the most used AGs were gentamicin, amikacin, tobramycin and netilmicin (Zarb, 2012). Due to the increase in prevalence of MDR Gram-negative infections (Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp.) there is renewed interest in AGs in human medicine and they were identified by the AMEG as critically important in the EU to treat these infections and enterococcal endocarditis, in addition.

Recommendations

*Proposal on categorisation for consideration by AMEG*

- Considering the AMEG criteria, veterinary-authorised AGs would be placed in Category 2 given (i) their importance in human medicine and (ii) the high potential for transmission of resistance

determinants between animals and humans and the potential for co-selection of resistance as described by the AMEG. However, according to the CVMP, AGs have a lower risk profile compared to fluoroquinolones and 3rd- and 4th-generation cephalosporins as they are used for a lower absolute number of individuals affected by all diseases for which these antimicrobials are one of few therapies available, and they are used less often for other infections than 3rd- and 4th-generation cephalosporins and fluoroquinolones in human medicine (WHO). Without precluding the AMEG decision, it is recommended that veterinary-authorised AGs could be placed in Category 2, although the AMEG could give consideration to a further stratification of the categorization.

- Those AGs that are not authorised for use in veterinary medicine would remain in the AMEG's category 3, pending risk assessment.

*Considerations for Marketing Authorisations and SPCs*

- The rationale for the indications for some VMPs containing fixed combinations of AGs, or combinations with antimicrobials from other classes, is questionable. In particular, this is the case for combinations including (dihydro)streptomycin as there is widespread resistance to this molecule in many bacterial species. The indications for (dihydro)streptomycin mono- products and AG combinations should be reviewed.
- The need for prolonged treatment durations (beyond 7 days) for certain products administered orally to groups of animals should be reviewed in the context of the specific indications.
- In reference to the above two recommendations and the scope of any referral procedures, review of groups of products would be prioritised according to risk.
- Based on the high levels of resistance to (dihydro)streptomycin and spectinomycin in many animal isolates, it should be recommended that use of these substances in particular is based on susceptibility testing.

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

*Needs for research*

- Further research should be conducted into the PK/PD surrogate indices which are predictive of clinical efficacy and enable optimisation of dosing regimens for AGs that are administered parenterally.
- Susceptibility testing should be standardised and veterinary clinical breakpoints should be established for AGs to enable the proper interpretation of susceptibility tests.
- The same AG resistance genes have been found in isolates from animals and humans and the potential for transmission of resistance from animal to humans is regarded as high. Further research is needed to elaborate on the link between the use of AGs in animals and the impact on public health.

Responsible parties: EURL-AMR, EFSA, VetCAST

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# 1. Background

Aminoglycosides (AGs), introduced in 1944, are among the oldest classes of antimicrobials. AGs have an aminocyclitol nucleus linked to amino sugars through glycosidic bonds (Ramirez and Tolmasky, 2010). The first AG discovered was streptomycin, produced by *Streptomyces griseus* (Schatz and Waksman, 1944). Several years later, other AGs produced by *Streptomyces* spp. were found (kanamycin, spectinomycin, tobramycin, neomycin, apramycin). In 1966, gentamicin, produced by *Micromonospora purpura*, was discovered followed by sisomicin produced by *M. inyoensis.* The first semisynthetic molecules were developed in the 1970s e.g. amikacin, netilmicin, isepamicin, dibekacin and arbekacin (van Hoek et al., 2011). AGs that are derived from *Streptomyces* spp*.* are named with the suffix –mycin (e.g. streptomycin), whereas those derived from *Micromonospora* spp*.* are named with the suffix –micin (e.g. gentamicin). The AGs can be divided into 4 groups: derivates containing the aminocyclitol streptidine (e.g. streptomycin, dihdrostreptomycin); derivates containing the aminocyclitol streptamine (spectinomycin), derivates containing a 4,5-disubstituted deoxystreptamine moiety (neomycin) and derivates containing a 4,6-disubstituted deoxystreptamine moiety (gentamicin, kanamycin, amikacin, tobramycin). The aminocyclitol spectinomycin is closely related to the aminoglycosides and will be discussed together with the AGs in this reflection paper.

AGs are bactericidal antibiotics that act by impairing bacterial protein synthesis through binding to the 30S ribosomal subunit (Dowling, 2013). AGs must penetrate into the bacterium to assert their effect and the uptake of AGs in the bacterial cell is an oxygen dependent process. Therefore, the spectrum of action of AGs is limited to aerobic and facultative anaerobic bacteria under aerobic conditions. AGs are less potent in hyperosmolar environments or environments with low pH. In addition, purulent debris at the infection site can bind to AGs and inactivate them (Dowling, 2013). AGs are hydrophilic molecules and relatively insoluble in lipids. They are poorly absorbed from the gut and penetration of the blood brain barrier is minimal (Dowling, 2013; Nau et al., 2010). The spectrum of activity includes Gram-negative bacteria, staphylococci, mycobacteria and leptospira. They have poor efficacy against streptococci and anaerobic bacteria and bacteria with intracellular location (e.g. severe or invasive salmonellosis). Enterococci generally show a degree of intrinsic resistance to AGs due to impermeability of the cell wall. Penetration into the bacterial cell can be enhanced by drugs that interfere with cell wall synthesis like beta-lactam antibiotics. Therefore, AGs are often used in combination with beta-lactams. This combination also broadens the spectrum of activity (Dowling, 2013).

In April 2013, the European Commission (EC) requested advice from the European Medicines Agency (EMA) on the impact of the use of antibiotics in animals on public and animal health and measures to manage the possible risk to humans. This reflection paper is based on the recommendation from the Antimicrobial Advice ad hoc Expert Group (AMEG) for further risk profiling of AGs to enable them to be placed within the AMEG's categorisation. The objective of the reflection paper is therefore to critically review the current knowledge on the usage of AGs, resistance development and the potential impact of this resistance on animal and human health.

# 2. The use of aminoglycosides in veterinary medicine

AGs are extensively used in veterinary medicine (EMA/ESVAC, 2016). They are used in different animal species, including both food producing animals and companion animals (Table 1). The substances reported to the ESVAC project as sold are amikacin, apramycin, (dihydro)streptomycin, framycetin, gentamicin, kanamycin, neomycin, spectinomycin and paromomycin. It must be noted that for amikacin no MRLs have been established and it can therefore not be used in food-producing animals. Paromomycin is approved in some Member States (MS) for treatment of colibacillosis in pigs and calves and has been used for the prevention of histomoniasis in turkeys (Kempf et al., 2013). Since 1976, AGs have not been authorised as growth promoters in the EU MSs. Before 1976, neomycin and hygromycin-B were authorised to be added to poultry feed for growth promotion only on a national level in certain MS (Castanon, 2007). In EU MS, AGs can therefore be employed only for clinical purposes. The most frequent use is therapy for septicemias, and infections of the digestive tract, respiratory tract and urinary tract in many animal species including cattle, pigs, poultry, sheep, goats, horses, dogs and cats. The use of the more toxic AGs such as neomycin is largely restricted to topical or oral therapy, while less toxic AGs such as gentamicin are also used for parenteral treatment. In addition, they are used off label as impregnated beads or regional perfusion to treat musculoskeletal infections in companion animals and horses. In particular gentamicin is indicated for *Pseudomonas aeruginosa* infections with few alternative treatments available (Dowling, 2013).

## *Route of administration and dosing*

AGs are used for parenteral, oral and topical applications.

Substances used for **parenteral applications** are (dihydro)streptomycin, gentamicin, kanamycin, framycetin, spectinomycin and neomycin. They are applied for therapy of blood stream infections as well as for infections of the gastrointestinal, respiratory tract and urinary tract in many animal species. Because of the unfavorable resistance situation and the risk of potential adverse reactions the use of (dihydro)streptomycin as mono-preparation is not recommended. (Dihydro)streptomycin in combination with penicillins is available as suspensions for intramuscular (i.m.) and subcutaneous (s.c.) administrations in cattle, pigs, horses, cats and dogs. Dosing regimens are 10-25 mg/kg once daily for 3 to 5 days or twice, 48 hours apart. Kanamycin is used i.m., s.c. or intravenously (i.v.) in dogs, cats, cattle, sheep, pigs and horses at dosages of 5-10 mg/kg, 3 to 4 times daily over a period of 3 to 4 days. Gentamicin is administered by i.m., s.c. or i.v. injection to dogs, cats, cattle, pigs and horses at dosages of 3-6.6 mg/kg over 3 to 5 (and in certain cases up to 10) consecutive days. Gentamicin is commonly administered twice daily on the first day and treatment is continued once daily from the second day onward. In young animals, the recommend dose is reduced by half. Framycetin is used in cattle at a dose of 5mg/kg i.m. twice daily for 3 days. Spectinomycin combined with lincomycin is administered i.m. to dogs, cats, horses, cattle and pigs at dosages of 10-20 mg/kg once or twice daily over 3 to7 days. Spectinomycin is administered as mono-substance to calves at dosages of 20-30 mg/kg i.m. on 3-7 days. Neomycin in combination with penicillins is used i.m. in cattle, sheep, pigs, horses, dogs, cats at a dose of 5-10mg/kg for 3 days (Löscher et al., 2014; Veterinary Medicines Directorate, website, last accessed 2017b; Vetidata, 2016; VMRI, 2016).

The majority of **oral formulations** (oral solution, oral powder, premix) are used for treatments in pigs, calves, sheep (lambs), poultry and rabbits. They are administered in a once daily treatment regimen as oral drenches (neonates) or in feed or drinking water/ milk over a period of 3-5 (and in exceptional cases even 7) days. Individual products are authorized for considerably longer treatment durations e.g. apramycin for 21 days or up to 28 days. Twice daily dosing regimens are used for

products containing neomycin in combination with sulfadiazine or streptomycin. AG doses vary depending on the substance and the target animal species intended to treat. For neomycin the daily dose is 10-75 mg/kg, for apramycin 4-80 mg/kg, for paromomycin 25-50 mg/kg and for gentamicin 1.1-3.4 mg/kg. In the context of a referral procedure under Article 35 of Directive 2001/82/EC (EMEA/V/R/A/110) and the subsequent commission decision, indications and posology of products containing a combination of spectinomycin and lincomycin to be administered orally to pigs and/or poultry were restricted to: pigs: 3.33 mg lincomycin and 6.67 mg spectinomycin/kg twice daily, for 7 days for the treatment and metaphylaxis of porcine proliferative enteropathy (ileitis) caused by *L. intracellularis*, and associated enteric pathogens (*E. coli*). The dose for chickens is 16.65 mg lincomycin and 33.35 mg spectinomycin/kg twice daily for 7 days for the treatment and metaphylaxis of chronic respiratory disease (CRD) caused by *Mycoplasma gallisepticum* and *E. coli*, and associated with a low mortality rate.

**Local applications** include ear drops, eye drops, topical application to the skin and intramammary and intrauterine preparations.

## *Animal species*

**Poultry**: In the EU neomycin, apramycin, spectinomycin and streptomycin are authorised for use in poultry (FIDIN, website, last accessed 2016; Norwegian Medicines Agency, 2003; Veterinary Medicines Directorate, website, last accessed 2017a). Outside the EU, gentamicin is used as subcutaneous injection in day-old chicks or in-ovo injections. In-ovo injection is a route for administration of Marek's disease vaccination in the U.S. and to prevent bacterial contamination of eggs, injection of gentamicin in combination with the vaccine is used (Bailey and Line, 2001). In-ovo injections or other applications of gentamicin in poultry are, however, not authorised in the EU as no MRLs for gentamicin for poultry exist. Neomycin and apramycin are authorised for oral treatment of enteric infections in poultry, e.g. for the treatment of *Escherichia coli* and Salmonella infections in young chickens, however antimicrobials are not permitted to be used for the specific purpose of control of Salmonella, with certain exceptions (Commission Regulation (EC) No. 1177/2006).

**Pigs**: In pigs, apramycin, gentamicin, paromomycin and neomycin are used for oral treatment of colibacillosis and salmonellosis (Norwegian Medicines Agency, 2012). Dihydrostreptomycin in combination with benzylpenicillin is authorised for respiratory infections caused by *Actinobacillus pleuropneumoniae* and/or *Pasteurella multocida* and for the treatment of Glässer's disease caused by *Haemophilus parasuis.*

**Cattle:** Neomycin, streptomycin, kanamycin and framycetin, in combination with other antimicrobial agents, are used in preparations for intra-mammary administrations to cows with mastitis. Neomycin and apramycin are used in calves for the treatment of bacterial enteritis caused by *E. coli* and Salmonellae. Gentamicin is used against respiratory infections of *Mannheimia haemolytica* and *Pasteurella multocida* in calves. Dihydrostreptomycin or streptomycin is used in the treatment of leptospirosis in cattle, swine and dogs. In non-ruminating calves paromomycin is used for the treatment of enteric infections caused by *E. coli*.

**Horses:** AGs (amikacin, neomycin and gentamicin) are mainly used for treatment of bacterial septicaemia, respiratory tract infection e.g. pneumonia, peritonitis, osteomyelitis, meningitis, wound infections, endometritis, often in combination with other antibiotics like beta-lactams. Topical application is recommended for infections of the eye and uterus. Amikacin is authorized in some MS for horses that are kept as companion animals and do not enter the food chain.

**Companion animals**: Injections of gentamicin or amikacin are licensed for the treatment of septicemia and respiratory infections. In textbooks AGs are recommended for the treatment of bacterial peritonitis, metritis, osteomyelitis, leptospirosis and nocardiosis (Dowling, 2013). AGs such as gentamicin, neomycin and framycetin are used as topical treatment for infections of the eye (blepharitis, conjunctivitis, keratoconjunctivitis, anterior uveitis), ear (otitis externa) and skin (FIDIN, website, last accessed 2016; Veterinary Medicines Directorate, website, last accessed 2017a).

Some products containing AGs, especially those with old marketing authorisations, are recommended for the treatment of "infections caused by susceptible organisms" in various animal species (FIDIN, website, last accessed 2016)

**Combination preparations:** AGs are often used in combination with other antimicrobials in order to achieve a synergistic effect or to broaden the spectrum of activity, such as with beta-lactams. Streptomycin and neomycin are authorised in the EU in combination with penicillin for treatment of a broad range of non-specific indications in livestock and companion animals (Veterinary Medicines Directorate, website, last accessed 2017a).

AGs are used in combination with beta-lactams and/or other antimicrobials in intramammary preparations. Common combinations for intramammary preparations for cows include neomycin/lincomycin, neomycin/streptomycin/penicillin, streptomycin/framycetin/penethamate, neomycin/penicillin, streptomycin/penicillin with or without nafcillin and neomycin/streptomycin/novobiocin/penicillin, among others.

Neomycin or (dihydro)streptomycin in combination with a beta-lactam is utilised for infections of the respiratory tract, digestive tract, nervous system and skin in various animal species. Neomycin/penicillin and streptomycin/penicillin combinations are licensed for the treatment of various infectious diseases in horses, sheep, pigs, dogs and cats caused by bacteria sensitive to the combination (Veterinary Medicines Directorate, website, last accessed 2017a). In pigs, spectinomycin/lincomycin combinations are used for the treatment of enzootic pneumonia, *Actinobacillus pleuropneumoniae* infections, porcine proliferative enteritis (*Lawsonia intracellularis*) and swine dysentery (FIDIN, website, last accessed 2016). In poultry, spectinomycin/lincomycin is applied for the treatment and prevention of chronic respiratory disease caused by *Mycoplasma gallisepticum* and *Escherichia coli* (Veterinary Medicines Directorate, website, last accessed 2017a). In the UK, a neomycin/streptomycin combination is used for prophylactic treatment in neonatal lambs, as an aid to prevention of enteric infection including watery mouth (enterotoxaemia caused by *E. coli*) and for the treatment of neomycin and streptomycin sensitive enteric infections in neonatal lambs (Veterinary Medicines Directorate, website, last accessed 2017a). The rationale for some of these combinations is disputable. Due to the widespread resistance of many bacterial species to streptomycin, streptomycin-penicillin combinations have very limited extra value. In addition, a synergistic effect of this combination has been shown for only a limited number of pathogens.

**Other applications of AGs**: certain AGs are used as anthelmintics in animals (destomycin A, hygromycin B). Furthermore, paromomycin, ribostamycin and streptomycin are used in horticulture as they have antifungal activity (Lee et al., 2005). Gentamicin is utilised as sperm diluter (Price et al., 2008) and as an antimicrobial preservative for vaccines. AGs are applied in apiculture, aquaculture and in other minor species such as rabbits, reptiles and birds, although safety and efficacy has not been established in all cases.

327 Table 1. Use of AGs in veterinary medicine

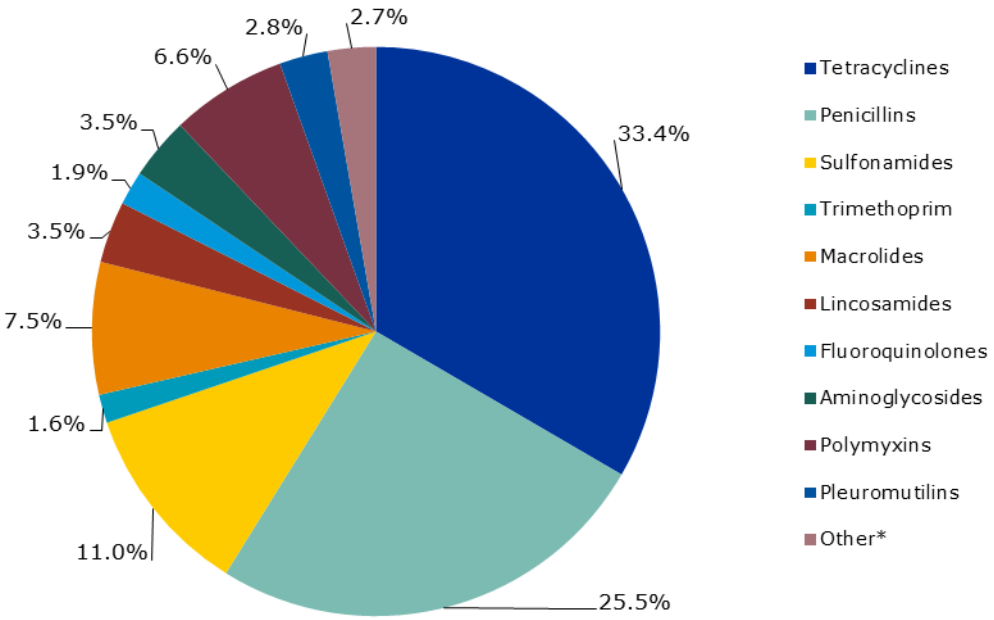
| Substance | Volume of use (2014) (ESVAC[1]) | Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume of sales | Duration of use | Species | Disease |
|---|---|---|---|---|---|
| kanamycin | < 2 tonnes | Two thirds parenteral and one third local sales.<br>Some small sales for oral use. | 3-4 days | Cattle | Gram-negative mastitis<br>Septicaemia<br>Respiratory infections<br>Urogenital infections |
| gentamicin | 12 tonnes | Two thirds parenteral, about one third oral.<br>Some sales for local use. | Injection 3-5 days | Pigs<br><br>Calves<br>Horses<br>Companion animals | Enteric infections<br>Respiratory infections<br>Septicaemia<br>Metritis<br>Ear, eye infections |
| amikacin | < 1 tonne | All parenteral | | Horses | Septicaemia (foals)<br>Metritis |
| apramycin | 21 tonnes | Mostly oral, small parenteral use. | In DW (drinking water) 5-7 days,<br>In-feed, up to 28 days | Poultry<br>Pigs<br>Calves | Enteric infections<br>Enterobacteriaceae |
| tobramycin | No sales reported | Topical | | Dogs | Eye infections caused by *Pseudomonas* spp. |
| streptomycin | 7 tonnes | Two thirds oral, about one third parenteral.<br>Some local use. | Injection 3 days | Poultry<br>Cattle, pigs, sheep,<br>Horses<br>dogs | Leptospirosis |
| dihydrostreptomycin | 129 tonnes | Mostly parenteral use, small oral use. Some local use. | Injection 3-5 days | Poultry<br>Pigs<br>Calves | Respiratory infections<br>Enteric infections<br>Gram negative mastitis |
| spectinomycin | 70 tonnes | Four fifths oral sales, one fifth parenteral sales. | In DW 7 days<br>Injection | Poultry<br>Pigs<br>Calves | Enteric infections<br>Respiratory infections |

[1] EMA/ESVAC, 2016, unpublished data.

| Substance | Volume of use (2014) (ESVAC[1]) | Major routes of administration in veterinary medicine by pharmaceutical form (oral, parenteral, local) and proportion of volume of sales | Duration of use | Species | Disease |
|---|---|---|---|---|---|
| | | | 3-7 days | | |
| paromomycin | 18 tonnes | Mostly sales for oral use, small amount sold for parenteral use. | Oral in DW 3-5 days | Pigs<br>Calves<br>Poultry | Enteric infections (Enterobacteriaceae,, cryptosporidium)<br>Histomoniasis (turkeys). |
| framycetin | < 1 tonne | For parenteral and local use | Injection 3 days | Cattle<br>Dogs | Gram negative mastitis<br>Ear infections |
| neomycin | 155 tonnes | Mostly sales for oral use, small sales for parenteral use.<br>Some small sales for local use | Oral 3-5 days<br>Injection 3 days | Poultry<br>Pigs<br>Horses<br>Lambs, goats<br>Cattle<br>Companion animals | Enteric infections (Enterobacteriaceae)<br>Septicaemia<br>Ear, eye infections |

In 2014, sales of AGs as percentage of the total sales for food-producing species (including horses), in mg/PCU, aggregated by 29 EU countries was 3.5 %. They are the 6$^{th}$ most common antimicrobial class used after tetracyclines, penicillins, sulfonamides, macrolides and polymyxins (Figure 1) (EMA/ESVAC, 2016).

**Figure 1.** Sales of antimicrobial agents by antimicrobial class as percentage of the total sales for food-producing species (including horses), in mg/PCU, aggregated by 29 European countries, for 2014 (EMA/ESVAC, 2016)




* Amphenicols, cephalosporins, other quinolones (classified as such in the ATCvet system).

There are marked differences in the sales of AGs between the different EU countries, being lowest in the Scandinavian countries and highest in Spain (Figure 2); these differences are not explained by the differences in overall antimicrobial use between countries in all cases.

**Figure 2.** Spatial distribution of veterinary sales of AGs (amikacin, apramycin, (dihydro)streptomycin, framycetin, gentamicin, kanamycin, neomycin) for food-producing animals in mg/PCU in 29 European countries for 2014 (EMA/ESVAC, 2016). Sales of spectinomycin and paromomycin are not included as they are reported under 'other antimicrobials' in the ESVAC report.


In the EU, approximately half of AG use is in oral forms (premix, oral powder or soluble in drinking water) and about half is as injectables (Figure 3 and Figure 4) (EMA/ESVAC, 2016). The most frequently used AGs are neomycin, dihydrostreptomycin and spectinomycin (Figure 5). Other substances from the group used in food producing species (where maximum residue limits (MRLs) have been established) are: apramycin, gentamicin, kanamycin, paromomycin, neomycin, framycetin and streptomycin. Renal accumulation of AGs results in detectable drug residues for prolonged periods of time and impacts on the withdrawal periods to be applied.

**Figure 3.** Distribution of veterinary sales by pharmaceutical form for AGs, in mg/PCU, by country, for 2014 (EMA/ESVAC, 2016)

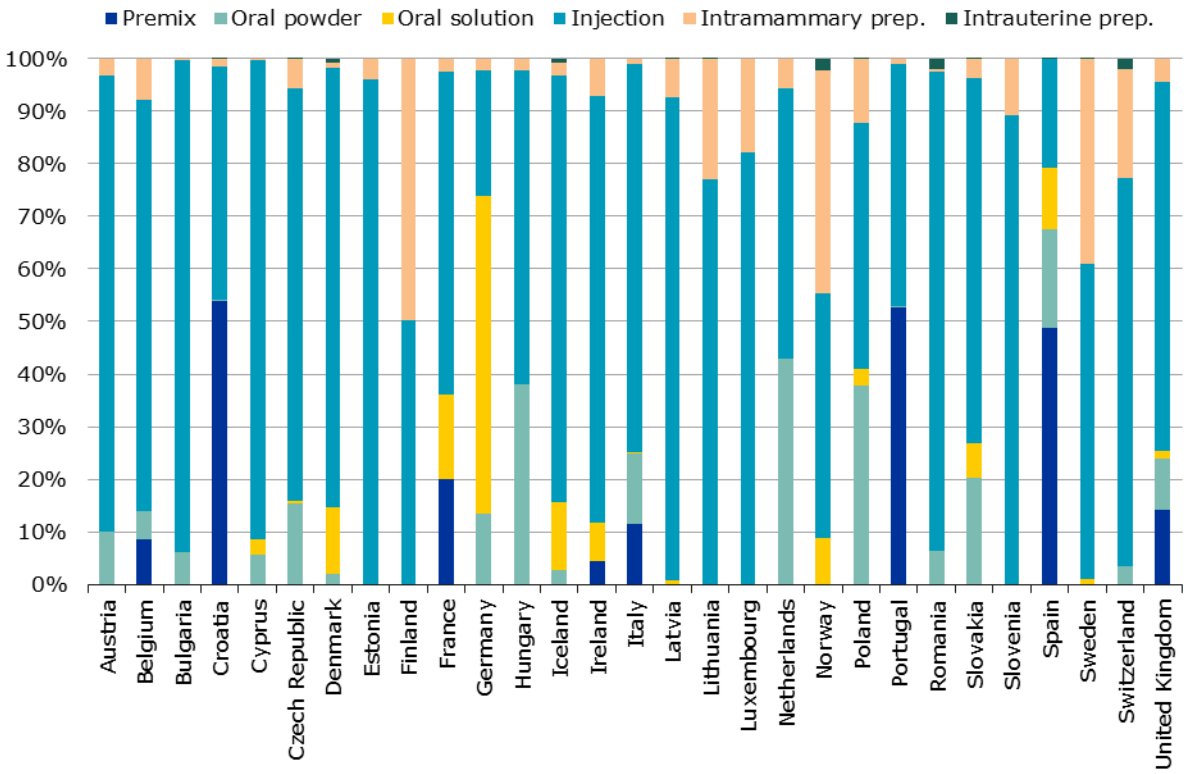
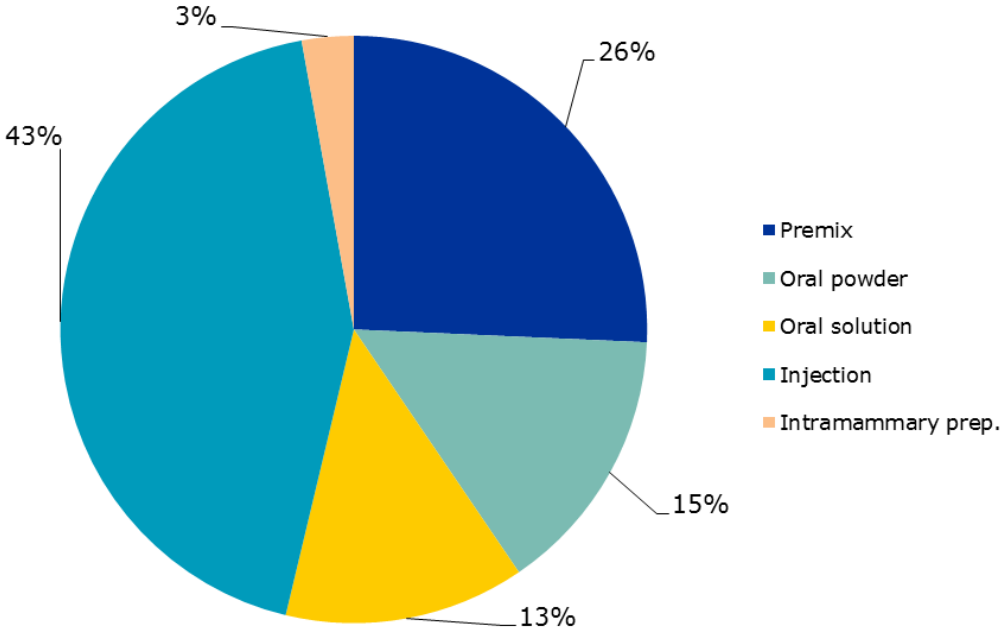
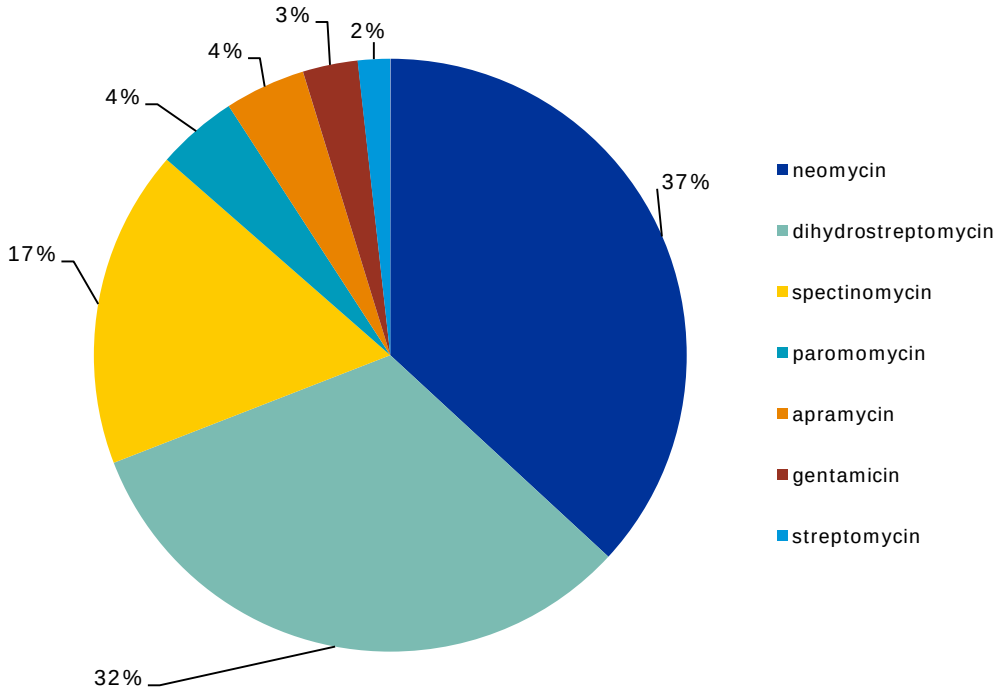



**Figure 4**. Distribution of veterinary sales by pharmaceutical form for AGs, for food-producing animals (including horses), in mg/PCU, aggregated by 29 European countries, for 2014 (ESVAC, as available in the Interactive Database). Sales of spectinomycin and paromomycin are not included.




In addition, 0.1% of the aminoglycosides were sold as intrauterine preparations.

**Figure 5.** Sales of aminoglycosides, spectinomycin and paromomycin in food-producing species, in percentage of total mg/PCU, aggregated for 29 European countries in 2014 (ESVAC, unpublished data)




Minor sales (≤0.5%) of kanamycin, framycetin and amikacin were also reported in 2014.

## *PK/PD relationship and dosing regimens*

To date, no specific PK/PD concepts are established for AGs in veterinary medicine. Knowledge on relationships between PK/PD parameters and clinical outcome of AGs derives from experience in human medicine, although laboratory animals have served as *in vivo* models for human PK/PD considerations (Andes and Craig, 2002).

For concentration-dependent antimicrobial agents, optimal dosing involves administration of high doses with long dosing intervals (Dowling, 2013). PK/PD indices have been proposed from *in vitro* and *in vivo*-infection models and subsequently validated in retrospective or prospective human clinical trials (Toutain et al., 2002). Two PK/PD indices Cmax/MIC (maximum concentration in serum or plasma/MIC) and 24-h AUC/MIC (area under the curve) are the most important PK/PD predictors for bacteriological and clinical efficacy of concentration-dependent antimicrobials (Craig, 1995; Jacobs, 2001; Tulkens, 2005).

Most authors have proposed the $C_{max}$/MIC ratio as the PK/PD index of choice for AGs (gentamicin, tobramycin, amikacin). A $C_{max}$/MIC ratio of 10 was best related to clinical outcome of patients with pneumonia caused by aerobic Gram-negative rods and with bacteremia caused by *Pseudomonas aeruginosa* (Kashuba et al., 1999; Moore et al., 1987; Zelenitsky et al., 2003). Besides a $C_{max}$/MIC ratio of 10-12 was determined to minimize the survival and overgrowth of resistant strains (Toutain et al., 2002). If this preferable peak to MIC ratio is obtained, most bacteria die within a short time, and consequently the effect of the time of drug exposure is minimal. Accordingly, in neutropenic and non-neutropenic models of infection, significantly more animals survived a potentially lethal challenge of bacteria when treated with a large dose of an AG rather than with the same dose given on an 8-hour

schedule. A high-dose and infrequent administration of AGs has also been shown to reduce the rate of nephrotoxicity (Ambrose et al., 2000). These findings and meta-analyses of different dosing regimens of AGs led to a shift in clinical dosing in humans from TID or BID to once a day treatments (Frimodt-Møller, 2002; Tulkens, 2005). The actual goal of AG therapy is to maximize peak concentrations to increase efficacy and reduce toxicity, to administer once-a-day and to reduce treatment duration as much as possible (Van Bambeke and Tulkens, 2011).

In veterinary medicine, the situation is more complex because of potential interspecies differences in pharmacokinetics and pharmacodynamics as well as differences in indications and target pathogens (Toutain, 2002). Besides, in animals AGs are to a large extent administered via the oral route for the treatment of gastrointestinal infections (Figure 4) where they exert their antibacterial activity in situ without being absorbed. Thus, for veterinary purposes human derived PK/PD concepts cannot be applied for oral applications at all and may be applied for parenteral applications by approximation, only.

When given via the parenteral route AGs were traditionally administered every 8-12 hours. Newer studies in veterinary patients support likewise high-dose, once daily therapy with AGs to avoid adaptive resistance and to reduce risks of toxicity. However, the optimal doses and the ideal drug monitoring strategy are still unknown. Dosages have to be modified in neonates and in animals with impaired liver or kidney function (Dowling, 2013).

In conclusion, prolonged treatment (longer than 7 days) should be avoided in order to reduce the risk of antimicrobial resistance. Dosing regimens, especially those for parenteral treatment, should be re-investigated.

# 3. The use of aminoglycosides in human medicine

Aminoglycosides are used primarily in infections involving aerobic, Gram-negative bacteria, such as *Pseudomonas*, *Acinetobacter*, and *Enterobactereriaceae.* Tobramycin, gentamicin, amikacin and netilmicin are used systemically for hospital acquired infections and *Pseudomonas* infections. Gentamicin, tobramycin, neomycin and paromomycin are used for topical application (Agence française de sécurité sanitaire des produits de santé, 2012). Kanamycin and amikacin are utilised for treatment of tuberculosis; streptomycin is rarely used. Amikacin may also be used against non-tuberculous mycobacterial infections.

In Belgium, the most applied AGs in hospitals are amikacin, gentamicin, and tobramycin (Ingenbleek et al., 2015). The most common route of administration for systemic infections is parenteral, by intravenous or intramuscular injection. Oral administration is limited to decontamination of the gut prior to surgery or in intensive care units, as bioavailability following oral administration is low (Huttner et al., 2013).

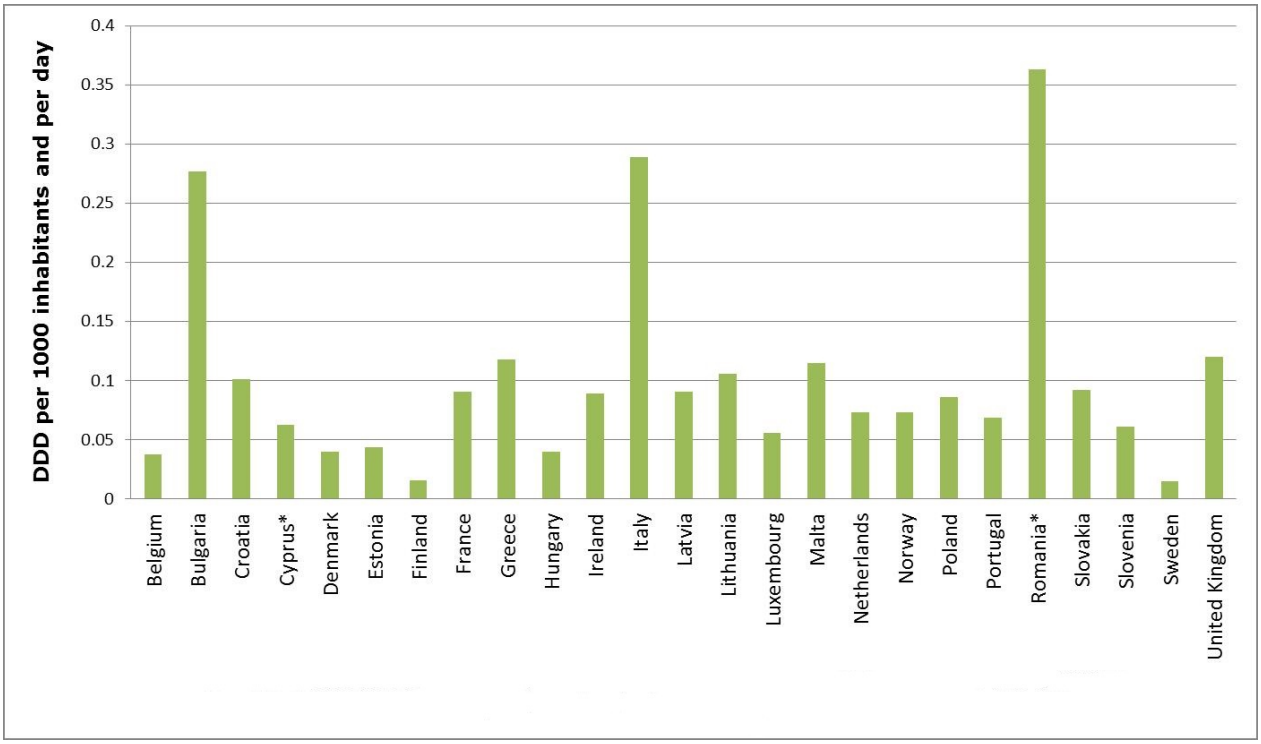
AGs are used for empirical treatment of sepsis, respiratory tract infections, urinary tract infections and some central nervous infections if multidrug-resistant Gram-negative bacteria are suspected to be involved (Poulikakos and Falagas, 2013). In addition, in combination with a beta-lactam or a glycopeptide, they are applied for the treatment of endocarditis caused by Gram-positive cocci. Enterococci are intrinsically resistant to low to moderate levels of AGs, but synergism is generally seen when they are combined with a cell-wall-active antimicrobial agent. Other applications are treatment of multidrug resistant tuberculosis and infections caused by Gram-negative pathogens, particularly *Enterobacteriaceae* (except for *Salmonella* spp.) and *Pseudomonas* spp. Streptomycin was the first AG to be used against tuberculosis, but is nowadays used less often due to high rates of

resistance and because it has to be used parenterally and the duration of therapy is usually long. As a second line of defence, kanamycin and amikacin are used to treat multidrug-resistant tuberculosis infections which are resistant to the front-line drugs isoniazid, rifampicin, and the fluoroquinolones (Labby and Garneau-Tsodikova, 2013). AGs are first line treatment for plague, brucellosis and tularaemia (Jackson et al., 2013). Aerosolized tobramycin, amikacin and gentamicin are used to treat *Pseudomonas* infections in patients with cystic fibrosis (Brodt et al., 2014; Jackson et al., 2013). Topical applications of various AGs are utilised for the treatment of ear infections and cutaneous leishmaniasis (Poulikakos and Falagas, 2013). Paromomycin is used to treat AIDS patients suffering from cryptosporidiosis (Fichtenbaum et al., 1993) and is an alternative against different parasites (amoebiasis, giardiasis) and sometimes used topically for the treatment of leishmaniasis. Spectinomycin is occasionally used for the treatment of gonorrhoea in patients allergic to penicillins (Table 2).

According to the ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals JIACRA (ECDC/EFSA/EMA, 2015), sales of AGs for animal use in 26 countries were 290.8 tonnes, while sales of AGs for human use during the same period were 4.7 tonnes. (5.2 mg/PCU animals and 0.2 mg/PCU for humans based on data from the JIACRA report.)

In the European Surveillance of Antimicrobial Consumption (ESAC) survey, including data from 20 European countries, details on the consumption of individual AGs are not reported separately. Available ESAC-Net data from 2015, however, show that there a large differences in AG consumption between MS, AG consumption being highest in Romania (0.363 DDD per 1000 inhabitants), Italy (0.289 DDD per 1000 inhabitants) and Bulgaria (0.277 DDD per 1000 inhabitants) whereas consumption is much lower in other countries, e.g. in Sweden (0.015 DDD per 1000 inhabitants) and Finland (0.016 DDD per 1000 inhabitants) (Figure 6). In a study applying ESAC-Net data and describing outpatient parenteral antibiotic treatment, out of antimicrobial classes given by the parenteral route, AGs were the second most commonly used (25.27%) after the cephalosporins (44.58%). Among the individual molecules gentamicin (18.53%) was more administered than the individual cephalosporins (e.g. ceftriaxone, 17.85%; cefazolin 13.16%) (Coenen et al., 2009).

**Figure 6.** Total consumption of aminoglycosides expressed as DDD per 1000 inhabitants in European countries in 2015




* Country provided only total care data.

Source: ESAC-Net (website, last accessed 2017)

Consumption data from European countries as outlined above are collected by continuous surveillance data (ECDC, 2014b) aggregated per country, although many countries have their own surveillance programme (DANMAP, 2013; NETHMAP, 2013). Long term monitoring in the Netherlands showed a doubling of occurrence of treatment with AGs in the Netherlands both in primary (from 0.02 to 0.04 DDD/1000 inhabitant-days) and hospital care (from 2.1 to 3.9 DDD/1000 inhabitant-days) during the last decade, similar to observations in ambulatory care in Belgium (RIZIV, 2011). In other European countries (e.g. Norway) this is not observed (NORM/NORM-VET, 2014). Large teaching hospitals tend to have the highest use (Ingenbleek et al., 2015; NETHMAP, 2013).

In addition to continuous surveillance as performed by the European Surveillance of Antimicrobial Consumption survey in outpatients and the hospital sector, targeted point prevalence surveys are done in hospitals (PPS HAI & AB) and long term care (HALT). Latest data show that on average 34.6% of patients receive antimicrobial therapy in acute care hospitals (Zarb et al., 2012) versus 4.4% in long term care facilities (LTCF) (HALT II) (ECDC, 2014a). Of these, the proportion of AG use was 4.5% and 1.2%, respectively. Considering the agents used in acute care, the most used AGs were gentamicin 3.7%, amikacin 1.1%, tobramycin 0.4%, netilmicin 0.1% (Zarb et al., 2012).

481 Table 2. Importance of AGs in human medicine

| 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 | Hazard of resistance transfer between animals and humans |
|---|---|---|---|
| kanamycin | Rarely used, not for first line treatment, MDR infections including tuberculosis | low | *M. tuberculosis* is of limited zoonotic relevance |
| gentamicin | Gram-negative infections, enterococcal and streptococcal endocarditis, brucellosis, tulaeremia, plague, oral decolonisation, impregnated beads to prevent surgical site infections | high | Enterobacteriaceae – high risk of horizontal transfer of resistance genes Enterococci – limited zoonotic risk |
| amikacin | MDR Gram-negative infections, MDR tuberculosis, Nocardia infections | high | *M. tuberculosis* is of limited zoonotic relevance |
| apramycin | No target | Not used | Selects for gentamicin resistance in *E. coli* |
| tobramycin | Gram-negative infections, Pseudomonas infections in cystic fibrosis | high | Enterobacteriaceae – high risk of horizontal transfer of resistance genes Enterococci – limited zoonotic risk |
| (dihydro)streptomycin | MDR tuberculosis, but very rarely used | low | *M. tuberculosis* is of limited zoonotic relevance |
| spectinomycin | Gonorrhoea in patients allergic to penicillins | low | Gonorrhoea is not transmitted to humans from non-human sources<br>Transfer of resistance genes from non-human sources unlikely |
| paromomycin | Cryptosporidiosis | low | *C. parvum* is of zoonotic relevance |

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# 4. Resistance mechanisms

Following extensive use of AGs in humans, food-producing animals and companion animals, resistance has emerged. Resistance occurs through several mechanisms. Resistance genes can be located on the chromosome, gene cassettes, plasmids, transposons or other mobile elements (Ramirez et al., 2013).

The three main mechanisms of bacterial resistance to AGs are the reduction of the intracellular concentration of the antibiotic, the enzymatic modification of the drug and the modification of the molecular target (Ramirez and Tolmasky, 2010). Resistance mechanisms are complex and differ between the AG molecules and between bacterial species, and generally there is less cross resistance when compared to other classes of antimicrobials. Many resistance genes are located on mobile elements increasing the likelihood of spread of AG resistance as well as co-resistance to other compounds (Ramirez and Tolmasky, 2010).

**Decreased intracellular concentration** can result from either reduced drug uptake or from active efflux mechanisms. Reduced uptake can occur in mutants deficient of components of the electron transport chain and has been described in *Pseudomonas* spp., *E.coli* and *S. aureus* (Taber et al., 1987). Gentamicin resistance by inactivation of an outer-membrane porin, which serves as an entry of gentamicin to the bacterial cell, has also been described (Poole, 2005).

AG efflux is a significant mechanism in *Pseudomonas* spp., *Burkholderia* spp., and *Stenotrophomonas spp.*, but has also been described in other bacteria such as *E. coli*, *Lactococcus lactis* and *Acinetobacter baumanii*. There are five families of efflux systems: the major facilitator superfamily (MF), the ATP-binding cassette family (ABC), the resistance-nodulation division family (RND), the small multidrug resistance family (SMR), and the multidrug and toxic compound extrusion family (MATE). The majority of AG transporters belong to the RND family (Poole, 2005). Genes encoding for AG efflux mechanisms are most often located on the chromosome, but members of the major facilitator superfamily (MF) can also be located on plasmids. Inhibitory as well as sub-inhibitory AG concentrations can lead to resistance. The ability of bacteria to survive antibiotic challenge without mutation is called adaptive resistance and can be caused by a decreased transport of the drug into the bacterial cell (Dowling, 2013). Adaptive resistance of *P. aeruginosa* has been shown to be associated with the overproduction of the RND efflux system MexXY-OprM (Hocquet et al., 2003). The clinical significance of adaptive resistance is that frequent dosing or constant infusion is less effective than high-dose, once daily administration as AGs act in a concentration-dependent manner (Dowling, 2013).

**Enzymatic drug modification.** Roberts et al. (2012) give an overview of most acquired resistance genes. A few novel spectinomycin resistance genes in staphylococci have been discovered since then (Jamrozy et al., 2014; Wendlandt et al., 2014; Wendlandt et al., 2013d). Resistance genes for AG modifying enzymes are often found on mobile elements. The most common mechanism of resistance to AGs in clinical isolates is the production of AG modifying enzymes such as acetyltransferases (AAC), phosphotransferases (APH) and nucleotidyltransferases (ANT) (Potron et al., 2015; Roberts et al., 2012; van Hoek et al., 2011). These enzymes modify the AG at the hydroxyl- or aminogroups of the 2-deoxystreptamine nucleus or the sugar moieties preventing ribosomal binding. Within the three major classes of modifying enzymes, a further subdivision can be made based on the target site of the enzymes (Roberts et al., 2012). To date, there are four acetyltransferases: AAC(1), AAC(2′), AAC(3), and AAC(6′); five nucleotidyltransferases: ANT(2″), ANT(3″), ANT(4′), ANT(6), and ANT(9); and seven phosphotransferases: APH(2″), APH(3′), APH(3″), APH(4), APH(6), APH(7″), and APH(9) (Roberts et al., 2012). Occasionally several subtypes of these enzymes are present in bacteria. The ACC enzymes are mainly found in Gram-negative bacteria such as Enterobacteriaceae, *Acinetobacter* spp. and *Pseudomonas* spp. They can, however, also be found in Gram-positive bacteria such as *Mycobacterium*

spp., *Streptomyces* spp., and *Enterococcus* spp. In addition, the bifunctional enzyme AAC(6')-APH(2'') can acetylate and subsequently phosphorylate its substrate. This enzyme has been found in *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Lactobacillus* spp.. The substrate profile of AAC(1) enzymes include neomycin, apramycin and paromomycin and that of AAC(2') enzymes include gentamicin, kanamycin, tobramycin, netilmicin, and dibekacin. Enzymes of subclass AAC(3)-I confer resistance to fortimicin, sisomicin and gentamicin, while those of subclass AAC(3)-II confer resistance to gentamicin, tobramycin, sisomicin, netilmicin, and dibekacin. AAC(6') enzymes are by far the most common acetyltransferases and cause resistance to gentamicin and sometimes amikacin. AAC(6')-Ib-cr is an enzyme that also confers resistance to selected fluoroquinolones such as ciprofloxacin (Ramirez and Tolmasky, 2010) (Table 3).

The ANTs represent the smallest class of AG inactivating enzymes. These enzymes catalyze the reaction between Mg-ATP and AGs to form the O-adenylated antibiotic molecule. To date, there are five classes of ANTs categorized depending on the position of adenylation on the AG molecule (Ramirez and Tolmasky, 2010). The ANT(2") and ANT(3") enzymes are more frequent among Gram-negative bacteria, whereas the ANT(4'), ANT(6), and ANT(9) enzymes are most often found in Gram-positive bacteria (Ramirez and Tolmasky, 2010; Shaw et al., 1993). The genes coding for all of these enzymes are often located on mobile genetic elements. ANT(6) enzymes have streptomycin as their substrate. The *ant(6)* gene is often found in a cluster *ant(6)-sat4-aph(3')-III* that specifies resistance to AGs and streptothricin. ANT(9) cause resistance to spectinomycin. ANT(4') enzymes confer resistance to tobramycin, amikacin and isepamicin. ANT(2″) mediates resistance to gentamicin, tobramycin, dibekacin, sisomycin and kanamycin. ANT(3") are the most commonly found ANT enzymes. They specify resistance to spectinomycin and streptomycin (Ramirez and Tolmasky, 2010).

APHs catalyze the transfer of a phosphate group to the AG molecule. They are widely distributed among bacterial pathogens and are encoded by genes usually found on multidrug resistance plasmids and transposons (Ramirez and Tolmasky, 2010). APH(2") plays an important role in Gram-positives resistant to gentamicin. APH(3')-IIIa, generally found in Gram-positive bacteria, confers resistance to a broad range of AGs including neomycin, paromomycin, kanamycin and amikacin, but not tobramycin or gentamicin. Isolates carrying APH(3) group enzymes show a resistance profile most often including kanamycin, neomycin and paromomycin, and APH(3') also to amikacin. APH(3'') mediates resistance to streptomycin. APH(4) mediates resistance to hygromycin and is not clinically relevant. APH(6) enzymes confer resistance to streptomycin. APH(7") mediates resistance to hygromycin. APH(9) enzymes confer resistance to spectinomycin (Ramirez and Tolmasky, 2010).

**Target modification.** Target-site modification naturally occurs in AG-producing bacteria: the bacterium protects the target by employing enzymes that add a methyl group to specific nucleotides in the 16S rRNA that are essential for AG binding, thus, inhibiting the antibiotic action without interfering with other ribosomal functions. This mechanism was described mainly in different species of the AG-producing genera *Streptomyces* and *Micromonospora*. Nowadays, the methylation of the ribosomal target responsible for high-level AG resistance is an emerging mechanism of great concern in clinically relevant Gram-negative bacteria. The first plasmid-mediated gene identified was the 16S rRNA methylase *armA* (Galimand et al., 2003). To date nine additional genes encoding methylases have been reported: *rmtA, rmtB, rmtC, rmtD, rtmD2, rmtE*, *rmtF*, *rmtG* and *npmA* (Potron et al., 2015). The genes encoding these determinants are usually located on mobile genetic elements and have been associated with genes coding for resistance to other antibiotic classes, such as quinolones (Qnr proteins) or β-lactam antibiotics (acquired AmpC-β-lactamases or extended-spectrum β-lactamases (ESBLs)). Recently these methyltransferases have been found in association with carbapenemases such as NDM-1 (Hidalgo et al., 2013b; Ho et al., 2011). The genes (rmtA, rmtB, rmtC, rmtD, rtmD2,

rmtE, rmtF, rmtG) confer resistance to gentamicin, tobramycin, kanamycin and amikacin whereas npmA confers resistance to gentamicin, tobramycin, kanamycin, amikacin, neomycin and apramycin, but not to streptomycin (Garneau-Tsodikova and Labby, 2016; Wachino and Arakawa, 2012).

Resistance to various AGs in staphylococci can be mediated by the genes *aacA/aphD* (kanamycin/gentamicin/tobramycin/amikacin resistance), *aadD* (kanamycin/neomycin/tobramycin resistance), *aphA3* (kanamycin/neomycin/amikacin resistance), *apmA* (apramycin resistance and decreased susceptibility to gentamicin) (Feßler et al., 2011; Wendlandt et al., 2013a), and *aadE* or *str* (streptomycin resistance) (Wendlandt et al., 2013b; Wendlandt et al., 2013c). Spectinomycin resistance in staphylococci is mostly mediated by spectinomycin 9-O-adenyltransferase encoded by the *spc* gene located on a transposon. Resistance in staphylococci to spectinomycin can also be due to the plasmid-associated gene *spd* and the chromosomal- or plasmid-located gene *spw* (Jamrozy et al., 2014; Wendlandt et al., 2013d).

AG resistance in Enterobacteriaceae mainly relies on the AG-modifying enzymes (APH, ANT and AAC). As mentioned before, AG efflux is a significant mechanism in *P. aeruginosa.* In *Acinetobacter baumannii*, the *armA* gene, located on a transposon, is widespread in many countries worldwide (Potron et al., 2015). In addition, *rmtB* has recently been identified in nine *A. baumannii* isolates in Vietnam (Tada et al., 2013).

In *Mycobacterium tuberculosis*, mutations in the genes *rpsL* and *rrs* encoding the ribosomal protein S12 and the 16S rRNA, respectively, are responsible for most of the high-level streptomycin resistance. The *rrs* A1401G is the most frequent mutation conferring amikacin and kanamycin resistance (Cohen et al., 2014). Overexpression of the AG acetyltransferase-encoding gene, *eis*, has mainly been associated with resistance to kanamycin. EIS is a unique enzyme capable of acetylating multiple positions of any given AG scaffold (Chen et al., 2011). This overexpression resulted from either point mutations in the promoter region of the *eis* gene or mutations of the *whiB7* gene, which encodes a putative regulator of the *eis* gene (Sowajassatakul et al., 2014). Although *eis* has been mainly associated with kanamycin resistance, resistance to amikacin has also been reported (Cohen et al., 2014). The gene *gidB*-when mutated- was found to be associated with low-level streptomycin resistance (Spies et al., 2008). The *gidB* gene encodes a 7-methylguanosine methyltransferase that specifically modifies residues in the 16S rRNA (rrs). It is a nonessential gene, and loss-of-function mutations in *gidB* result in failure to methylate G527 within the 530 loop of the 16S rRNA molecule. Many different *gidB* mutations, including deletions are associated with AG resistance, suggesting that loss of function of this gene confers resistance (Cohen et al., 2014).

607 Table 3. Most relevant AG resistance genes and their spectrum of action

| Resistance gene | Aminoglycoside to which this gene confers resistance | Occurrence |
|---|---|---|
| **Acetylytranferases** | | |
| AAC (1) | neomycin, apramycin, paromomycin | *uncommon* |
| AAC (2') | gentamicin, tobramycin, kanamycin, netilmicin, dibekacin | *uncommon* |
| AAC (3) subclass I | gentamicin | *uncommon* |
| AAC (3) subclass II | gentamicin, tobramycin, netilmicin, dibekacin, sisomycin, kanamycin | *uncommon* |
| AAC (3) subclass III | gentamicin, tobramycin, netilmicin, neomycin | *uncommon* |
| AAC (3) subclass IV | gentamicin, tobramycin, (kanamycin), netilmicin, neomycin | *uncommon* |
| AAC (6') | (amikacin), gentamicin | *common* |
| **Phosphotransferases** | | |
| APH (2'') | gentamicin | *uncommon* |
| APH (2'')/ AAC (6') | gentamicin, tobramycin, kanamycin, (amikacin) | *common in Gram-positives* |
| APH (3') subclass I | kanamycin, neomycin, paromomycin | *common* |
| APH (3') subclass II | kanamycin, neomycin, paromomycin | *common* |
| APH (3') subclass III | kanamycin, neomycin, paromomycin, (amikacin) | *highly disseminated in Gram-positives* |
| APH (3'') | streptomycin | *common* |
| APH (6) | streptomycin | *uncommon* |
| APH (9) | spectinomycin | |
| **Nucleotyltransferases** | | |
| ANT (2') (synonym *aadB*) | gentamicin, tobramycin, kanamycin, dibekacin, sisomycin | *common in integrons* |
| ANT (3'') (synonym *aadA)* | streptomycin, spectinomycin | *very common* |
| ANT (4') (synonym *aadD, aad2*) | tobramycin, amikacin, isepamicin (dibekacin) | |
| ANT (6) (synonym *aadE*) | streptomycin | *very common* |
| ANT (9) (synomym *aad(9)* or *spc*) | spectinomycin | *uncommon* |
| **Methyltransferases** | | |
| armA | gentamicin, tobramycin, kanamycin, amikacin, | |
| rmtA, rmtB, rmtC, rmtD, rtmD2, rmtE, rmtF, rmtG | gentamicin, tobramycin, kanamycin, amikacin | *uncommon* |
| npmA | gentamicin, tobramycin, kanamycin, amikacin, neomycin, apramycin | *uncommon* |

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# 5. Consideration on susceptibility testing of aminoglycosides

Susceptibility data from national monitoring programs are available and MIC determination via broth microdilution is the most frequently used method in these programs. Methodologies used differ among countries as they use different standards and guidelines (EUCAST, CLSI or country-specific ones), different antimicrobial agents for the same bacteria, different concentration ranges for the same antimicrobial agent and different interpretative criteria (Schwarz et al., 2013). A standard defines specific and essential requirements for materials, methods and practices to be used in a non-modified form. In contrast, guidelines describe criteria for a general operating practice, procedure or material for voluntary use. A guideline can be used as written or can be modified by the user to fit specific needs. This hampers comparison of the results. *In vitro* susceptibility testing for many antimicrobials including AGs is problematic for many bacterial species, since standards and guidelines for determination of minimal inhibitory concentrations (MIC) do not include all micro-organisms. Single class representatives cannot be used for AGs as resistance is not a class effect, i.e. there are numerous resistance genes specifying a wide variety of resistance mechanisms with in part strikingly different substrate spectra. Resistance to streptomycin and spectinomycin for example is distinct from resistance to gentamicin, kanamycin and/or tobramycin (Schwarz et al., 2010). Counterwise, unrelated enzymes, affecting different sites, can confer the same resistance phenotypes. Despite these difficulties the enzymes produced by isolates can sometimes be predicted from susceptibility testing (Livermore et al., 2001).

To date, EUCAST has no veterinary-specific breakpoints. However, CLSI has veterinary-specific breakpoints for amikacin applicable to *E. coli*, and *P. aeruginosa* from dogs, foals, adult horses, *Staphylococcus* spp. from dogs, *S. aureus* form foals and adult horses, *Streptococcus* spp. from dogs, *Streptococcus equi* subsp*. zooepidemicus* and subsp. *equi* from foals and adult horses (CLSI, 2015a).

For *Enterococcus* spp. (*E. faecalis, E. faecium, E. gallinarum/E. casseliflavus*), aminoglycosides (except when tested positive for high-level resistance) may appear to be active *in vitro*, but are not effective clinically and should not be reported as susceptible. Anaerobic bacteria, such as *Clostridium* spp., *Bacteroides* spp. and *Fusobacterium canifelinum* are intrinsically resistant to AGs (CLSI, 2015b).

A recent study showed that results of susceptibility testing for gentamicin for *K. pneumoniae* resistant to carbapenems obtained with Vitek 2 and Etest should be interpreted with caution, especially if the EUCAST breakpoints were used. False gentamicin susceptibilities were observed using Vitek 2 and occurred with *K. pneumoniae* isolates carrying *armA* (Arena et al., 2015).

Susceptibility testing of *Pseudomonas* isolates against tobramycin using MALDI-TOF MS technology has been explored and was able to distinguish between resistant and susceptible isolates. Therefore, this technique has the potential to allow for the susceptibility testing of a much wider range of antimicrobial substances in the future (Jung et al., 2014).

# 6. Occurrence of resistance in bacteria from animals

## *6.1. Food-producing animals*

Generally, resistance to streptomycin is very common while resistance to the other AGs is detected less frequently. Resistance in Dutch Salmonella isolates was uncommon for gentamicin and kanamycin (2-3 %), but 31 % of the isolates were resistant to streptomycin. In Campylobacter isolates from pigs and poultry, resistance was very rare for gentamicin and neomycin (0-0.6 %), while the level of resistance to streptomycin was high (49 %). For *E. coli*, 2 %, 4 % and 34 % of the isolates were resistant to gentamicin, kanamycin and streptomycin, respectively and the resistance levels were highest in isolates from conventional broilers. For *Enterococcus* spp. the levels of resistance were high for streptomycin (30-43 %) and low for gentamicin (2 %). Reduced susceptible and resistant isolates were defined using epidemiological cut-off values (MARAN, 2014). In Denmark, porcine Salmonella isolates were often resistant to streptomycin (47 %), while resistance to gentamicin, apramycin and neomycin was rare (2-3 %). The level of resistance among Danish *Campylobacter jejuni* isolates to streptomycin and gentamicin was very low. The level of resistance to streptomycin and kanamycin among Enterococcus isolates was much higher for imported broiler meat than for Danish broiler meat (DANMAP, 2013). The level of AG resistant *E. faecalis* was higher in pigs than in broilers. The level of resistance of *E. coli* in Denmark was low in broilers and cattle for all AGs tested. In pigs, 42 % of *E. coli* isolates were resistant to streptomycin, while only 1-2 % of the isolates were resistant to gentamicin, apramycin and neomycin (DANMAP, 2013). In 2014 recommendations for the panel used for susceptibility testing by EFSA changed, excluding streptomycin, neomycin, apramycin and spectinomycin, depending on the bacterial species tested. Generally the levels of resistance to gentamicin of *E. coli*, enterococci, Campylobacter and Salmonella were low in 2014 and 2015 (DANMAP, 2015).

Data from 17 MS show that resistance to gentamicin in Salmonella isolates from *Gallus gallus* is generally low (5.9%), but there are big differences between MS: in most MS resistance to gentamicin was either not detected or low, but among the relatively large proportion of isolates from Romania, moderate levels of resistance to gentamicin (18.4 %) were reported, thus influencing the overall resistance levels. In addition, there are also differences between Salmonella species: in *S*. Kentucky (n=47) from *Gallus gallus* from Italy, Romania and Spain resistance to gentamicin was common, 64% of isolates being non-susceptible (EFSA/ECDC, 2015); in Salmonella isolates from turkey resistance to gentamicin was 8.8%, but in *S*. Kentucky the percentage of resistant isolates was as high as 85% (EFSA/ECDC, 2015). The percentage of Salmonella isolates resistant to gentamicin originating from cattle and pigs was generally very low. Resistance to gentamicin was not found in *Campylobacter jejuni* from broilers, whereas only 2.5% of *Campylobacter coli* isolates were gentamicin resistant. Levels of resistance to gentamicin was also low in *Campylobacter coli* isolates from pigs (1.9%) and *Campylobacter jejuni* isolates from cattle (0.9%) (EFSA/ECDC, 2015). Resistance to streptomycin was generally high in *E. coli* isolates from *Gallus gallus*, pigs and cattle (45.7%, 47.8% and 17.6% respectively), whereas resistance to gentamicin was low (6.4%, 1.8% and 2% respectively). In *Enterococcus faecium* and *E. faecalis* isolates resistance to streptomycin was relatively common (between 10% and 60%, depending on the animal and bacterial species), while resistance to gentamicin was rarely found (EFSA/ECDC, 2015).

Equine *E. coli* isolates were generally susceptible to gentamicin and the resistance rate was only 8.8 % (Schwarz et al., 2013). A significant increase in the percentage of *E. coli* isolates resistant to

gentamicin was identified in equine *E. coli* isolates from 2007-2012 (53.9 %) compared to isolates from 1999-2004 (28.5 %) (Johns and Adams, 2015).

Characterization of 227 *Streptococcus suis* isolated from pigs during 2010 - 2013 showed high level resistance to neomycin (70.0%) and gentamicin (55.1%) and resistance to AGs was attributed to aph(3')-IIIa and aac(6')Ie-aph(2")-Ia genes (Gurung et al., 2015; Schwarz et al., 2013). Integron-borne AG and sulphonamide resistance was found frequently among avian pathogenic *E. coli* (APEC) in Italy. High levels of resistance were observed for streptomycin (67.2%), whereas resistance against gentamicin (16.7%), kanamycin (14.7%), and apramycin (3.0%) was lower (Cavicchio et al., 2015).

Bovine *Pasteurella multocida* remain relatively susceptible to AGs with 60 %, 92 %, 90 % and 99 % of the isolates being susceptible to streptomycin, spectinomycin, neomycin and gentamicin, respectively. In France, 82 % of all *Mannheimia haemolytica* isolates were susceptible to spectinomycin and neomycin and 88 % to gentamicin. Coagulase-positive staphylococci isolated from the udder were often susceptible to all AGs tested, with 88 % to 99 % of the isolates susceptible to streptomycin, kanamycin, neomycin and gentamicin. Equine *E. coli* isolates were often resistant to streptomycin, with approximately half of the isolates being susceptible, whereas most *E. coli* isolates remained susceptible to amikacin, gentamicin, neomycin and kanamycin (76 %-100 % susceptibility). Among equine *S. aureus* isolates susceptibility to AGs was 88 % for kanamycin and 89 % for gentamicin and streptomycin (Anses, 2015). The emergence of 16SrRNA methylases in bacteria of animal origin was first discovered in Spain in 2005 in an *E. coli* isolate of pig origin harbouring the *armA* gene (Gonzalez-Zorn et al., 2005). Since then the same mechanism has been detected in *E. coli* isolates from pigs, chicken, and cows in different countries (Chen et al., 2007; Davis et al., 2010; Deng et al., 2011; Du et al., 2009; Hopkins et al., 2010; Liu et al., 2008). To date, 16SrRNA methylases do not appear to be common in veterinary bacteria in EU MS, but the use of most AGs would select for resistance as these enzymes result in resistance to almost all AGs, especially those of clinical relevance in humans.

Resistance to gentamicin, tobramycin and kanamycin was common (36%) among MRSA CC398 isolates collected from pigs at Dutch slaughterhouses (de Neeling et al., 2007). Non-susceptibility to gentamicin was also found among MRSA isolates on broiler farms (Wendlandt et al., 2013b). Non-susceptibility to gentamicin (40%), neomycin (30%) and amikacin (1%) was found among 1290 MRSA isolates from pigs, veal calves, poultry and meat in the Netherlands (Wagenaar and Van de Giessen, 2009). High prevalence of non-susceptibility to AGs has been reported in methicillin-susceptible *S. aureus* CC398 isolates (Vandendriessche et al., 2013). MRSA CC1 isolates from dairy cattle and humans in Italy were often kanamycin resistant and carried *aphA3* and *sat* (conferring streptothricin resistance) genes with Tn5405-like elements, and contained several markers indicating a human origin (Alba et al., 2015).

## *6.2. Companion animals*

According to data from Resapath (Anses, 2015), France, susceptibility percentages for feline *E. coli* were 59 % for streptomycin, 92 % for kanamycin, 97 % for gentamicin and 89 % for neomycin. Among coagulase-positive staphylococci originating from skin and muscular infections in dogs, 63 % and 59 % were susceptible to streptomycin and kanamycin respectively and 86 % were found susceptible to gentamicin. Susceptibilities of feline staphylococci were similar. Canine *E. coli* isolates were generally susceptible to gentamicin (> 90 % of isolates susceptible). In Germany, 96 % of canine and feline *S. aureus* isolates from ear infections and 84 % of *S. aureus* from skin infection were susceptible to gentamicin. Gentamicin susceptibility percentages for *S. pseudintermedius* isolates were 87 % for isolates from ear infections and 74 % for isolates from skin infections. Resistance in *P.*

*aeruginosa* isolates from ear infections of companion animals was found in 25 % of the isolates, while only 41 % of the isolates were fully susceptible (Schwarz et al., 2013). Among 103 methicillin-resistant *S. pseudintermedius* isolates from dogs originating from several countries in Europe, the USA and Canada resistance to gentamicin/kanamycin (88.3%), kanamycin (90.3%), streptomycin (90.3%) and streptothricin (90.3%) was very common (Perreten et al., 2010). Among clinical ESBL-producing Enterobacteriaceae from companion animals resistance to AGs was encoded by *aadA1* (29% of all isolates), *aadA2* (17%), *aadA4* (14%), *aac(6′)-Ib* (8%), *strA* (3%), *strB* (25%) and *ant2a* (8%) (Dierikx et al., 2012).

In Spain, seven *K. pneumoniae* ST11 isolates from dogs and cats were found to be resistant to AGs, and the ArmA methyltransferase was responsible for this phenotype (Hidalgo et al., 2013a). In China, the *rmtB* gene was detected in 69 out of 267 Enterobacteriaceae isolates collected from pets. The *rmtB* gene was commonly found with ESBL $bla_{\text{CTX-M-9}}$ group genes within the same IncFII plasmid (Deng et al., 2011).

# 7. Possible links between the use of AGs in animals and resistance in bacteria of animal origin

A systematic review on the effect of oral antimicrobials on antimicrobial resistance in porcine *E. coli* found that oral administration of AGs increased the prevalence of antimicrobial resistance (Burow et al., 2014). Sun et al. (2014) investigated the effect of treatment of sows with lincomycin, chlortetracycline and amoxicillin on resistance development of the intestinal microbiota. The treatment increased the abundance of AG resistance genes, probably due to co-selection. Apramycin and neomycin fed in subtherapeutic concentrations to pigs enhanced transfer of an antimicrobial resistance plasmid from commensal *E. coli* organisms to Yersinia and Proteus organisms in an infection model using isolated ligated intestinal loops (Brewer et al., 2013). Apramycin consumption at farm level in pigs was most probably driving the increasing occurrence of apramycin/gentamicin cross-resistant *E. coli* in diseased pigs and healthy finishers at slaughter in Denmark. The duration of use and amounts used both had a significant effect on the prevalence of apramycin/gentamicin cross-resistance in diseased weaning pigs at the national level (Jensen et al., 2006). Another Danish study investigated the effect of apramycin treatment on transfer and selection of a multidrug-resistant *E. coli* strain in the intestine of pigs and found that the use of apramycin may lead to enhanced spread of gentamicin-resistant *E. coli* (Herrero-Fresno et al., 2016). In a study investigating the influence of oral administration of a fluoroquinolone, an AG and ampicillin on prevalence and patterns of antimicrobial resistance among *E. coli* and *Enterococcus* spp. isolated from growing broilers, the overall resistance to all drugs tested reached the highest level among enterococci after medication with gentamicin. The frequency of resistance against most antimicrobials tested was significantly higher in *E. coli* isolated from broilers receiving intermittent antimicrobial pressure than that from non-medicated broilers (Da Costa et al., 2009). On a German broiler farm, resistance to spectinomycin in *E. coli* isolates increased significantly with age in all three production turns, despite the fact that the substances was not used on the farm. A possible explanation for this phenomenon was co-selection by the use of other antimicrobials (Schwaiger et al., 2013).

Selection of an ESBL plasmid conferring resistance not only to β-lactams but also to AGs, tetracycline, trimethoprim, sulfonamides, and erythromycin, as well as biocides and heavy metals occurred *in vitro* by the use of different antibiotics, including kanamycin at concentrations far below the MIC (Gullberg et al., 2014). These findings suggest that low concentrations of antibiotics present in polluted external environments and in the gut of exposed animals and humans could allow for selection and enrichment

of bacteria with multi-resistance plasmids and thereby contribute to the emergence, maintenance, and transmission of antibiotic-resistant disease-causing bacteria.

In conclusion, there is evidence that the usage of AGs in veterinary medicine is associated with the increased prevalence of resistance in bacteria in animals. Usage of AGs in humans is also associated with increased prevalence of resistance in humans. In human isolates from the Enterobacteriaceae family, there was a significant effect of selection pressure of gentamicin in the selection of resistant *K. pneumonia* and *E. coli* and amikacin in the selection for resistant *E. coli* and *E. cloacae* isolates (Sedláková et al., 2014). Another study showed that the abundance of antibiotic resistance genes more than doubled during selective digestive decontamination with colistin, tobramycin and amphotericin B in ICU patients, mainly due to a 6.7-fold increase in AG resistance genes, in particular aph(2″)-Ib and an aadE-like gene (Buelow et al., 2014).

# 8. Impact of resistance on animal health

AGs are important for the therapy of common infections and are widely used in food producing species and companion animals. They are categorised as veterinary critically important antibiotics by the OIE. Loss of efficacy of AGs could have a serious negative impact on animal health and welfare. Although AGs are very important antimicrobials for treatment of animal infections, they are seldom the sole alternative. In horses, gentamicin is one of the few options for Gram-negative infections. Alternative treatment options are trimethoprim/sulphonamide combinations (TMPS), 3$^{rd}$- and 4$^{th}$-generation cephalosporins and fluoroquinolones, but the latter two antimicrobials should also be used restrictively and resistance to TMPS is common among Gram-negative bacteria. In pigs, AGs are important drugs for the treatment of post-weaning diarrhoea. Alternatives are tetracycline, trimethoprim-sulphonamide combinations and ampicillin/amoxicillin, but the prevalence of resistance among *E. coli* to these antimicrobials is high. Other alternatives include colistin or quinolones. For Pseudomonas infections AGs are one of the few treatment options. In companion animals AGs are used to treat ear and eye infections caused by *Pseudomonas* spp. by topical application of drops or ointments. For such topical applications, alternatives include polymyxins and fluoroquinolones. For systemic treatment of Pseudomonas infections, fluoroquinolones are one of the few other treatment options and the use of this class of antimicrobials should be restricted to conditions were no alternative treatment options are available.

# 9. Impact of resistance on human health

All AGs (including streptomycin, neomycin and kanamycin), with the exception of the aminocyclitol spectinomycin, are categorized as “critically important” antimicrobials for human medicine by WHO, whereas spectinomycin is categorized as “important” as it is not the sole or one of the limited treatment options for a serious human disease nor is it used to treat diseases caused by either: (1) organisms that may be transmitted to humans from non-human sources or, (2) human diseases causes by organisms that may acquire resistance genes from non-human sources (ref WHO). AGs are most often used in combination with beta-lactams in the empirical treatment of a broad range of life-threatening infections in humans. Nephrotoxicity and ototoxicity and the discovery of less toxic antimicrobials in recent decades has limited the use of AGs in human medicine (Poulikakos and Falagas, 2013). High levels of resistance and multidrug-resistance in certain bacteria to other antimicrobials, however, have resulted in renewed interest in the AGs.

The increasing prevalence of multidrug-resistance in Gram-negative bacteria such as Enterobacteriaceae, *P. aeruginosa* and *A. baumannii* due to the accumulation of unrelated resistance

mechanisms (e.g. to β-lactams and AGs) has resulted in the development of new synthetic compounds(e.g. plazomicin), which are less susceptible to AG-modifying enzymes (Poulikakos and Falagas, 2013).

To date, extended-spectrum β-lactamases (ESBLs) conferring resistance to broad-spectrum cephalosporins, carbapenemases conferring resistance to carbapenems, and 16S rRNA methylases conferring resistance to all clinically relevant AGs are the most important causes of concern (Potron et al., 2015). In recent years, the global dissemination of Enterobacteriaceae, including *Salmonella* spp., that co-produce 16S-rRNA methylases and carbapenemeses such as NDM-1 metallo-β-lactamase (MBL) is becoming a serious threat to human health. The resistance genes are often co-located on the same plasmid. Although 16s rRNA methylases are mainly reported from human clinical isolates, *armA*, *rmtB* and *rtmC* have also been found in isolates from pets and farm animals (Wachino and Arakawa, 2012). In addition to 16s rRNA methylases, resistance to aminoglycosides in both Gram-positive and Gram-negative clinical isolates is often related to the production of modifying enzymes of several classes. In countries using an AG combined with penicillin as empirical treatment of sepsis, increasing resistance will result in a shift to applying more resistance-driving options and thereby lead to even more resistance. It should be noted, however, that a systematic review assessed mortality, treatment failures and antimicrobial resistance by comparing beta-lactam monotherapy versus any combination of a beta-lactam with an AG for human cases of blood stream infections. The authors concluded that the addition of an AG to beta- lactams for sepsis should be discouraged, since mortality rates were not improved and the addition of AGs considerably increased the risk for nephrotoxicity (Paul et al., 2006).

Furthermore, besides infections with multidrug-resistant Enterobacteriaceae, *Pseudomonas* spp. and *Acintobacter* spp., multidrug-resistant tuberculosis and enterococcal endocarditis are among the diseases for which availability of AGs is critically important due to few alternatives (EMA, 2014). For enterococcal endocarditis ampicillin combined with gentamicin has long been considered the regimen of first choice, but during the last decade the combination of ampicillin with ceftriaxone has been shown to be equally effective (Falcone et al., 2015).

In conclusion, AGs are important drugs for the treatment of infections with multidrug-resistant Gram-negative bacteria and multidrug-resistant tuberculosis, but they are seldom the only therapeutic option.

# 10. Transmission of resistance and determinants between animals and humans

According to the AMEG answers to the request for scientific advice on the impact on public health and animal health of the use of antibiotics in animals, there are three categories of antimicrobials: category 1 are antimicrobials used in veterinary medicine where the risk for public health is currently estimated low or limited, category 2 are antimicrobials where the risk for public health is currently estimated higher, category 3 includes antimicrobials currently not approved for use in veterinary medicine.

AGs are frequently used in veterinary and human medicine and resistance has emerged. Resistance can be due to chromosomal mutations, but resistance determinants are often located on mobile elements such as transposons, integrons and plasmids. The same resistance genes have been found in isolates from animals and humans (García et al., 2014; Wendlandt et al., 2013a; Wendlandt et al., 2013b). In addition, resistance to AGs has been found in bacteria that can cause foodborne infections in humans, such as *Salmonella* spp. and *Campylobacter* spp., although AGs are generally not used to treat Salmonella or Campylobacter infections in humans. Antibiotic resistance in several *Salmonella enterica* serovars is due to genomic islands carrying a class 1 integron, which carries the resistance genes. *Salmonella* genomic island 1 (SGI1) was found in *S. enterica* serovar Typhimurium DT104 isolates, which are resistant to ampicillin, chloramphenicol, florfenicol, streptomycin, spectinomycin, sulfonamides and tetracycline. Several *Salmonella* serovars have since been shown to harbor SGI1 or related islands. SGI1 is an integrative mobilizable element and can be transferred experimentally into *E. coli* (Hall, 2010). Co-selection to all these antimicrobials can potentially result from the use of AGs if SGI1 is present.

Livestock-associated MRSA CC398 (LA-MRSA) isolates from veterinarians in Belgium and Denmark were often resistant to gentamicin, kanamycin and tobramycin mediated by *aac (6')-aph(2a")* or *aadC* and LA-MRSA carriage was significantly associated with contact with livestock (Garcia-Graells et al., 2012). This indicates that LA-MRSA resistant to AGs can be transmitted between animals and humans.

In humans, AGs are mostly used for infections caused by bacteria that are not transmitted via food or contact with animals. Enterobacteriaceae and enterococci can, however, be transmitted between animals and humans. AGs are used for treatment of zoonotic infections such as tuberculosis, brucellosis and tularaemia. It should be noted that even bacteria causing human infections not directly linked to animals may acquire resistance determinants from bacteria with zoonotic potential. Recently carbapenem-resistant *P. aeruginosa* isolates, with additional resistances to all fluoroquinolones, AGs, β-lactams and some even non-susceptible to colistin, were found in Ohio. The isolates contained the metallo-betalactamase gene $bla_{VIM-2}$ within a class 1 integron. Genomic sequencing and assembly revealed that the integron was part of a novel 35-kb region that also included a Tn501-like transposon and Salmonella genomic island 2 (SGI2)-homologous sequences indicative of a recombination event between *Salmonella* spp. and *P. aeruginosa* (Perez et al., 2014). The indirect risk from the use of AGs in food animals should therefore be taken into account in determining risk profiles.

Extended-spectrum or plasmidic AmpC beta-lactamase producing Enterobacteriaceae are widely distributed among human and animal populations. Transmission of ESBL/pAmpC-*E. coli* from animals to humans can potentially occur by direct contact, through the food chain or the environment. Evidence for clonal transmission of ESBL-producing *E. coli* between humans and broilers was found on conventional broiler farms, and horizontal gene transfer was suspected on both conventional and organic farms (Huijbers et al., 2014; Huijbers et al., 2015). ESBL- and carbapenemase-encoding

plasmids frequently bear resistance determinants for other antimicrobial classes, including AGs and fluoroquinolones, a key feature that fosters the spread of multidrug resistance in Enterobacteriaceae (Ruppé et al., 2015).

The prevalence 16S rRNA methylase gene *rmtB* in Enterobacteriaceae isolates from pets in China was high. *rmtB* was detected in 69/267 isolates, most of which were clonally unrelated. The coexistence of the *rmtB* gene with the $bla_{CTX\text{-}M\text{-}9}$ group genes on the same plasmid was found (Deng et al., 2011). Although transmission between animals and humans was not studied, the location of resistance determinants on plasmids indicates that transmission could potentially occur.

The risk of transmission of multidrug-resistant tuberculosis from animals to humans is limited, as the main resistance mechanism for Mycobacteria is chromosomal mutation. In addition, tuberculosis in humans is mainly caused by *M. tuberculosis*, which is transmitted from humans-to-humans. Bovine tuberculosis is a reportable disease in EU MS and has been eradicated in many EU MS. During the years 2006–2012, the proportion of cattle herds infected or positive for *M. bovis* in the EU (all MSs) was at a very low level and ranging from 0.37 % in 2007 to 0.67 % in 2012 (EURL for Bovine Tuberculosis, website, last accessed: 2017).

Altogether, these data show that the probability of transfer of AG resistance from animals to humans is high (Table 4).

910 Table 4. Classification of AGs according to their probability of transfer of resistance genes and resistant bacteria

| Substance | Prevalence of resistance * | Mobile genetic element-mediated transfer of resistance[a] | Vertical transmission of resistance gene(s)[b] | Co-selection of resistance[c] | Potential for transmission of resistance through zoonotic and commensal food-borne bacteria[d] | Evidence of similarity of resistance: genes / mobile genetic elements / resistant bacteria[e] | Overall probability of resistance transfer |
|---|---|---|---|---|---|---|---|
| kanamycin, gentamicin, amikacin, apramycin, tobramycin, paromomycin, framycetin, neomycin | low | 3 | 3 | 3 | 3 | 3 | High |
| spectinomycin, (dihydro)streptomycin, | high | 3 | 3 | 3 | 3 | 3 | High |

911
912 [a]Mobile genetic element-mediated transfer of resistance. Defined as a resistance gene that is transmitted by means of mobile genetic elements (horizontal transmission of the gene occurs). Probability (1 to 3): 1, no gene mobilization
913 described; 2, gene is exclusively on the core bacterial chromosome; 3, gene is on a mobile genetic element, e.g. plasmid.

914 [b]Vertical transmission of resistance gene. Defined as the vertical transfer of a resistance gene through the parent to the daughter bacteria in a successful, highly disseminated resistant clone of bacteria through a bacterial population,
915 e.g. *E. coli* ST131 clone, MRSP CC(71) clone, MRSA ST398 clone. Probability (1 to 3): 1, no vertical transmission of gene described as associated with in a particular successful resistant clone; 2, gene is exclusively on the core bacterial
916 chromosome in a particular successful resistant clone; 3, gene is on a mobile genetic element, e.g. plasmid, in a particular successful resistant clone.

917 [c]Co-selection of resistance. Defined as selection of resistance which simultaneously selects for resistance to another antimicrobial. Probability (1 to 3): 1, no co-mobilization of the gene or risk factor described; 2, gene is either co-
918 mobilized or a risk factor has been described; 3, gene is co-mobilized and a risk factor has been described.

919 [d]Transmission of resistance through zoonotic and commensal food-borne bacteria. Defined as transmission of resistance through food-borne zoonotic pathogens (e.g. *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *E. coli* VTEC) or
920 transmission of resistance through commensal food-borne bacteria (e.g. *E. coli*, *Enterococcus* spp.). Probability (1 to 3): 1, no transmission of resistance through food-borne zoonotic pathogens or commensal food-borne bacteria; 2,
921 transmission of resistance through food-borne zoonotic pathogens or commensal food-borne bacteria; 3, transmission of resistance through food-borne zoonotic pathogens and commensal food-borne bacteria.

922 [e]Evidence of similarity of resistance: genes/mobile genetic elements/resistant bacteria. Genes - Defined as similar resistance gene detected in bacterial isolates of animal and human origin; Mobile genetic elements - Defined as a similar
923 resistance mobile genetic element detected in bacterial isolates of animal and human origin; Resistant bacteria - Defined as a similar bacterium harboring a resistance gene (either chromosomally or mobile genetic element-encoded) of
924 animal and human origin. Probability (1 to 3): 1, unknown resistance similarity; 2, genes or mobile genetic elements or resistant bacteria similar between animals and humans; 3, genes and mobile genetic elements similar between
925 animals and humans; 4, genes and mobile genetic elements and resistant bacteria similar between animals and humans.

926 * Based on surveillance data from foodborne pathogenic and commensal bacteria (EFSA/ECDC, 2017)

# 11. Discussion

AGs are bactericidal antibiotics that act by impairing bacterial protein synthesis. Many AGs are used in both veterinary and human medicine, except for apramycin, which is only used in animals. In European livestock and in companion animals, AGs are used for the treatment of a variety of different conditions.

In animals, AGs are administered orally, topically on the skin, as intramammary or intrauterine preparation, as ear or eye drops or as injectables. In veterinary medicine, the sales of AGs accounted for 3.5% of the total sales (in PCU) for food producing species from 26 EU/EEA member states in 2013. The the most commonly sold AGs were neomycin, dihydrostreptomycin and spectinomycin: together they accounted for 84% of the total sales of AGs, while sales of gentamicin account for only 3%.

In human medicine, AGs are used primarily in infections involving aerobic, Gram-negative bacteria, such as *Pseudomonas*, *Acinetobacter*, and Enterobacteriaceae and in combination with beta-lactams for the treatment of endocarditis caused by enterococci or streptococci. Newer AGs, such as gentamicin, amikacin and tobramycin, are more often used in EU MS, especially as injectables, while older AGs such as streptomycin are rarely used and neomycin is only used orally and for topical application.

AGs are concentration-dependent antimicrobial agents, and optimal parenteral dosing involves administration of high doses with long dosing intervals. Most injectable or oral products in veterinary medicine are administered for 3-5 days. Some products, however, are licensed for usage for more than 7 days, some for in-feed use even for 21 or 28 days. Treatment durations longer than 7 days and parenteral administrations more than once daily should be reviewed. Any indications for treatment of salmonella infections in chickens should be in line with EC regulations and take account of the public health risk.

Interpretation of susceptibility testing is impaired by the lack of veterinary breakpoints for most AGs. Veterinary breakpoints should therefore be established.

The amount of AGs used in animals as well as humans varies significantly for those EU/EEA countries for which there are data on consumption. Reasons for these differences are unknown in veterinary medicine, but the sales of AGs as a percentage of the total antimicrobial sales (mg/PCU) for food producing animals in 29 EU countries was just 3.5% in 2014.

The usage of AGs in animals and humans is associated with the occurrence of resistance. Resistance can be due to chromosomal mutations, but resistance determinants are more often located on mobile elements. Resistance can be transmitted between animals and humans through clonal transfer of pathogenic bacteria, e.g. Livestock associated -MRSA, *Salmonella* spp. or *Campylobacter* spp., but resistance genes can also be transferred horizontally on mobile elements between bacteria and even between different bacterial species. On these mobile elements, genes mediating resistance to different AGs and also to other classes of antimicrobials are often present, facilitating co-selection of AG resistance by the use of other antimicrobials. Resistance mechanisms are complex and differ between the AG molecules and also between bacterial species. Cross-resistance to several AGs by a single mechanism/plasmid does occur, but generally there is no complete cross resistance. The genes encoding resistance to AGs like streptomycin or spectinomycin are generally different from those of gentamicin or tobramycin. With some exceptions, resistance to streptomycin and spectinomycin is generally common in isolates from animals, including those with zoonotic potential, while resistance to gentamicin, amikacin and kanamycin is still uncommon.

Similar resistance genes and mobile elements have been found in bacteria from humans and animals. Resistance to AGs has been found in bacteria that can cause foodborne infections in humans, such as

*Salmonella* spp. and *Campylobacter* spp. as well as in potentially zoonotic bacteria such as (LA)-MRSA, although in humans these infections would not be generally treated with AGs; *E. coli* and enterococci, however, can also carry the same AG resistance genes and can be transmitted between animals and humans. AGs are used in humans for the treatment of *E. coli* and enterococcal infections. In addition, as resistance genes are often present on mobile genetic elements, they can potentially be transmitted from zoonotic bacteria to human pathogens, e.g. from Salmonella to Klebsiella or other Gram-negative bacteria. Therefore, the probability of transmission of AG resistance from animals to humans is regarded high. Although the prevalence of resistance depends on the bacterial species investigated and the EU MS, the use of AGs in food-producing animals may in general have an impact on human health. Since very few new and effective antimicrobials for the treatment of infections due to multidrug-resistant Gram-negative bacteria are likely to be launched in the near future, there is an urgent need to implement strategies that may slow down the development of acquired resistance (Potron et al., 2015).

Generally, the risk from oral products used mostly to treat enteric infections in pigs, chickens and calves (apramycin, neomycin, streptomycin, spectinomycin, gentamicin) is much higher, as these products are used as mass medication and as AGs are not absorbed from the gut, the gut flora is exposed to considerable selective pressure. Resistance to streptomycin is common in enteric indicator bacteria such as *E. coli* and Enterococcus species, but fortunately the percentage of resistance to gentamicin in these bacteria is still relatively low, most likely due to differences in the resistance mechanisms and differences in the amounts used in veterinary medicine.

The risk for the emergence of resistance in humans from the use of topical products including drops used to treat eye and ear infections (mainly Pseudomonas infections) in companion animals is generally regarded as low, as individual animals are treated and this local route of administration does not result in selective pressure on the gut flora. This also holds for the use of AGs as intramammaries (mainly neomycin; streptomycin and dihydrostreptomycin) for the treatment of mastitis in cattle, although the use of intramammaries as dry cow therapy might result in a somewhat higher risk as more individuals are treated (unless selective treatment is practised) and long acting preparations are used. The risk for the emergence of resistance in humans from the use of AGs (streptomycin, gentamicin) as injectables will generally be lower if animals are treated individually rather than as a group. The risk for humans will also be higher when gentamicin is used, as this AG is also commonly used in humans.

In veterinary medicine, AGs are one of the few treatment options for Pseudomonas infection and for infections with Gram-negative bacteria in horses.

In human medicine, AGs are important for the treatment of infections with *Pseudomonas* spp., *Acinetobacter* spp. and multidrug-resistant Enterobacteriaceae, however they are rarely the sole treatment option. The risk of transmission of resistant Enterobacteriaceae to humans from non-human sources is regarded high. AGs have been considered critical for humans as a sole or one of limited treatment options for enterococcal endocarditis. For enterococcal endocarditis and bacteriaemia, however, alternative treatment options are now available and there are studies indicating that mono-therapy with beta-lactams is as effective as combination therapy with AGs, with less toxicity for patients. Therefore, AGs are rarely the sole treatment option in human or veterinary medicine. In the AMEG report the potential risk level of AGs included consideration of the risk of transmission of resistant Enterococcus spp. and Enterobacteriaceae to humans from non-human sources. Molecular epidemiological studies based on multi-locus sequence typing (MLST) revealed that the vast majority of *E. faecium* isolates causing clinical infections and nosocomial outbreaks in humans belong to a globally dispersed polyclonal subpopulation, genotypically different from *E. faecium* strains colonising

animals and healthy humans in the community. There was a significant discrepancy in accessory gene content between hospital and community ampicillin-resistant *E. faecium* that includes putative virulence and antimicrobial resistance genes, and indicates that if zoonotic transfer occurs, it only occurs infrequently (de Regt et al., 2012). For *E. faecalis*, however, the same MLST types can be detected in isolates from food, animals and patients with clinical infections and therefore the zoonotic potential is higher (Hammerum, 2012). For *E. coli*, Salmonella species and LA-MRSA, the risk of transmission of resistance determinants between animals and humans is regarded high. AGs are also important for the treatment of multidrug-resistant tuberculosis. The risk of transfer of resistance between animals and humans is regarded low, as resistance in Mycobacteria is due to chromosomal mutations and most human cases in EU MS are caused by *Mycobacterium tuberculosis,* which is mainly transmitted from humans-to-humans. Bovine tuberculosis is rare in Europe overall.

If AGs were no longer available for veterinary medicine then it could be speculated that other antimicrobials would replace their use. Alternatives to AGs for the treatment of some multidrug-resistant Gram-negative infections in animals include antimicrobials that are critically important for the treatment of human infections, such as fluoroquinolones and colistin. For a complete risk assessment, the consequences of the use of these alternatives instead of AGs should also be taken into account, but this is beyond the scope of this reflection paper. In addition, as most AG resistance genes are located on mobile genetic elements which often also harbour genes mediating resistance to other classes of antimicrobials and thus facilitate co-selection, prudent use of all antimicrobials in human and veterinary medicine is of great importance.

# 12. Conclusion

Considering the AMEG criteria, veterinary-authorised AGs would be placed in Category 2 given (i) their importance in human medicine and (ii) the high potential for transmission of resistance determinants between animals and humans and the potential for co-selection of resistance as described by the AMEG. However, according to the CVMP, AGs have a lower risk profile compared to fluoroquinolones and 3$^{rd}$- and 4$^{th}$-generation cephalosporins as they are used for a lower absolute number of individuals affected by all diseases for which these antimicrobials are one of few therapies available, and they are used less often for other infections than 3$^{rd}$- and 4$^{th}$-generation cephalosporins and fluoroquinolones in human medicine (WHO). Without precluding the AMEG decision, it is recommended that veterinary-authorised AGs could be placed in Category 2, although the AMEG could give consideration to a further stratification of the categorization. Those AGs that are not authorised for use in veterinary medicine would remain in the AMEG's category 3 pending further risk assessment.

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