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

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Executive summary

Aminoglycosides (AGs) are important antibacterial agents for the treatment of various infections in humans and animals.

In 2015, AGs accounted for 3.5% of the total sales (mg/PCU) of antimicrobials used in food-producing species as reported from 30 European countries. The most frequently used AGs are neomycin, dihydrostreptomycin and spectinomycin. The most frequent uses are as 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. They are used for parenteral, oral and topical applications and approximately half of the total use is as oral formulations.

In human medicine, AGs, especially gentamicin, tobramycin, and amikacin, are used for treatment of infections caused by Gram-negative bacteria such as Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp., and Gram-positive bacteria such as enterococci and mycobacteria. They are mainly administered 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. 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* spp. and *Campylobacter* spp. 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* spp. and *Campylobacter* spp. isolates, is common. In livestock-associated MRSA CC398, resistance to gentamicin is commonly found. There is evidence that the use 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. The risk of transmission of AG resistance from animals to humans with regards to tuberculosis is regarded low as the resistance mechanism in mycobacteria is through chromosomal mutations and no horizontal transfer of a plasmid related mechanism has been reported to date. Clonal transmission of multidrug-resistant tuberculosis is mainly caused by *M. tuberculosis* which is transmitted from humans to humans.

AGs have been categorised as critically important antimicrobials both for human medicine by WHO and for veterinary medicine by OIE. AGs are, however, rarely the sole treatment option in either veterinary or human medicine.

The Antimicrobial Advice *ad hoc* Expert Group (AMEG) categorisation considers the risk to public health from AMR due to the use of antimicrobials in veterinary medicine. Considering the current AMEG criteria, all veterinary-authorised AGs, including streptomycin, would be placed in Category 2 (higher risk for public health) given (i) their importance in human medicine, (ii) the high potential for

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transmission of resistance determinants between animals and humans, and (iii) the potential for co-selection of resistance as described by the AMEG. According to the CVMP, use of AGs in veterinary medicine has a lower risk to human health compared to use of fluoroquinolones and 3rd- and 4th-generation cephalosporins as AGs are used for a lower absolute number of human patients affected by all diseases for which these antimicrobials are one of few therapies available, and are used less often for other infections than are 3rd- and 4th-generation cephalosporins and fluoroquinolones in human medicine (WHO). The AMEG is currently reviewing the criteria of its categorisation and could give consideration to its further stratification.

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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 four groups: derivates containing the aminocyclitol streptidine (e.g. streptomycin, dihydrostreptomycin); derivates containing the aminocyclitol streptamine (spectinomycin); those containing a 4,5-disubstituted deoxystreptamine moiety (neomycin) and finally 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 antimicrobials 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 their uptake in the bacterial cell is an oxygen dependent process. Therefore, the spectrum of activity 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 of AGs includes Gram-negative bacteria, staphylococci, mycobacteria and leptospira. They have poor efficacy against streptococci, anaerobic bacteria and intracellular bacteria. Enterococci and streptococci generally show a degree of intrinsic resistance to AGs due to impermeability of their cell wall, but penetration into the bacterial cell can be enhanced by other antimicrobials that inhibit cell wall synthesis such as the 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 antimicrobials 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, 2017). 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 in European countries are amikacin, apramycin, (dihydro)streptomycin, framycetin, gentamicin, kanamycin, neomycin, spectinomycin and

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paromomycin. It must be noted that for amikacin no MRLs have been established and therefore it cannot 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 septicaemias, 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 and for joint injections to treat septic arthritis in horses. In particular gentamicin is indicated for *Pseudomonas aeruginosa* infections with few alternative treatments available (Dowling, 2013).

2.1. 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 administered for treatment of bloodstream infections, as well as for infections of the gastrointestinal, respiratory and urinary tract in many animal species. Due to the unfavourable resistance situation the use of (dihydro)streptomycin as mono-preparation without susceptibility testing 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, three to four times daily over a period of three to four 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 three to five (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. Following a referral to CVMP (EMEA/V/A/104), it was concluded that the dose of gentamicin for adult horses should be 6.6 mg/kg once daily for 3-5 days; use was not recommended in foals due to safety concerns. Framycetin is used in cattle at a dose of 5 mg/kg i.m. twice daily for three 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 three to seven days. Spectinomycin is administered as a mono-substance to calves at dosages of 20-30 mg/kg i.m. for three to seven days. Neomycin in combination with penicillins is used i.m. in cattle, sheep, pigs, horses, dogs and cats at a dose of 5-10mg/kg for three days (Löscher et al., 2014; Veterinary Medicines Directorate, website, last accessed 2018b; 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 (to neonates) or in feed or drinking water/milk over a period of three to five (and in exceptional cases even seven) days. Individual products are authorised 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 for treatment. For neomycin the

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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. For pigs the dose is 3.33 mg lincomycin and 6.67 mg spectinomycin/kg twice daily, for seven 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 seven 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. See below.

2.2. Animal species

Poultry: In the EU, neomycin, apramycin, spectinomycin and streptomycin are authorised for use in poultry (FIDIN, website, last accessed 2018; Norwegian Medicines Agency, 2003; Veterinary Medicines Directorate, website, last accessed 2018a). Outside the EU, gentamicin is used as a 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 the eggs, gentamicin is injected in combination with the vaccine (Bailey and Line, 2001). *In-ovo* injections or other applications of gentamicin in poultry are not authorised in the EU as no MRLs for gentamicin exist for poultry. Neomycin and apramycin are authorised for oral treatment of enteric infections in poultry, e.g. for the treatment of *Escherichia coli* and *Salmonella* spp. infections in young chickens. Antimicrobials are not permitted to be used for the specific purpose of control of *Salmonella* spp., with certain exceptions (Commission Regulation (EC) No. 1177/2006). It is noted that colibacillosis is essentially a systemic infection in poultry and it therefore appears contrary that it should be treated orally with substances such as apramycin that are considered to have very low oral bioavailability (Afifi and Ramadan, 1997).

Pigs: In pigs, apramycin, gentamicin, paromomycin and neomycin are used for oral treatment of colibacillosis and salmonellosis (Norwegian Medicines Agency, 2012). Aminoglycosides are an important alternative to colistin for the treatment of post-weaning diarrhoea caused by *E. coli* in pigs (FVE, 2017). 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 commonly used for treatment of bacterial septicaemia, respiratory tract infection (e.g. pneumonia), peritonitis, osteomyelitis, meningitis, wound infections, joint infections and endometritis, often in combination with other antimicrobials such as beta-lactams. For infections in neonatal foals, where Gram-negative bacteria are isolated from a high proportion of cases and a bactericidal effect is desirable, there is a preference to select

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aminoglycosides (gentamicin or amikacin) as empirical therapy whilst awaiting culture results (Giguère and Afonso, 2013). Topical application is recommended for infections of the eye and uterus (Dowling, 2013). Amikacin is authorised 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 2018; Veterinary Medicines Directorate, website, last accessed 2018a).

Gentamicin and tobramycin are effective topically to manage otitis externa caused by *P. aeruginosa* (Barnard and Foster, 2017). AGs are included for the treatment of multidrug resistant infections in treatment guidelines for respiratory infections in dogs and cats (Lappin et al., 2017) and for deep pyoderma, as third-line choice (Beco et al., 2013). They are also suggested in DSAVA guidelines (DSAVA, 2015) to treat ESBL-producing *E coli* urinary tract infections when there is resistance to authorised alternatives.

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 2018)

Combination preparations: AGs are often used in combination with other antimicrobials, such as beta-lactams, in order to achieve a synergistic effect or to broaden the spectrum of activity. 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 2018a).

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, amongst others.

Neomycin or (dihydro)streptomycin in combination with a beta-lactam are 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 2018a). In pigs, injectable spectinomycin/lincomycin combinations are used for the treatment of respiratory infections and arthritis caused by Mycoplasmata (FIDIN, website, last accessed 2018) and a premix is used for treatment of porcine proliferative enteropathy (ileitis) caused by *Lawsonia intracellularis* (Veterinary Medicines Directorate, website, last accessed 2018a). In poultry, spectinomycin/lincomycin is used for oral treatment and metaphylaxis of chronic respiratory disease caused by *Mycoplasma gallisepticum* and *E. coli* (Veterinary Medicines Directorate, website, last accessed 2018a). 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 2018a). The rationale for some of these combinations is disputable. Due to the widespread resistance of many bacterial species to streptomycin, streptomycin-

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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 and only *in vitro*. The penicillin/streptomycin combination was withdrawn from the US market in 1993 and later by Australia as no evidence of clinical synergy was presented (Gloyd, 1992; NRA, 1999).

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). Streptomycin is also used to control fire blight caused by *Erwinia amylovora* in orchards (Stockwell and Duffy, 2012). 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 with use being "off-label".

Table 1. Use of AGs in veterinary medicine

| Substance	Volume of use (2015) (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 of sales for parenteral use and one third for local use. Low amounts of sales for oral use.	3-4 days	cattle	Gram-negative mastitis septicaemia respiratory infections urogenital infections
gentamicin	11 tonnes	Two thirds parenteral, about one third oral use. Some sales for local use.	Injection 3-5 days	pigs calves horses companion animals	enteric infections respiratory infections septicaemia metritis ear, eye infections
amikacin	< 1 tonne	All parenteral use.		horses	septicaemia (foals) metritis
apramycin	23 tonnes	Mostly oral, small amount of parenteral use.	In DW (drinking water) for 5-7 days In-feed, up to 28 days	poultry pigs calves	enteric infections Enterobacteriaceae
tobramycin	no sales collected	Topical use.		dogs	eye infections caused by *Pseudomonas* spp.
streptomycin	12 tonnes	Two thirds oral use, about one third	Injection 3 days	poultry cattle,	enteric infections, leptospirosis

Substance	Volume of use (2015) (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
		parenteral. Some local use.		pigs, sheep, horses dogs	Gram-negative mastitis
dihydrostreptomycin*	111 tonnes	Mostly parenteral use, small amount for oral use. Some local use.	Injection 3-5 days	poultry pigs cattle	respiratory infections enteric infections Gram-negative mastitis
spectinomycin	66 tonnes	Four fifths oral sales, one fifth parenteral sales.	In DW 7 days Injection 3-7 days	poultry pigs calves	enteric infections respiratory infections
paromomycin	14 tonnes	Mostly sales for oral use, small amount sold for parenteral use.	Oral in DW 3-5 days	Pigs Calves Poultry	enteric infections (Enterobacteriaceae, *Cryptosporidium* spp.) histomoniasis (turkey)
framycetin	< 1 tonne	For parenteral and local use	Injection 3 days	cattle dogs	Gram-negative mastitis ear infections
neomycin	131 tonnes	Mostly sales for oral use, small amounts sold for parenteral and local use.	Oral 3-5 days, injection 3 days	poultry pigs horses lambs, goats cattle companion animals	enteric infections (Enterobacteriaceae) septicaemia ear, eye infections

- EMA/ESVAC, 2016, unpublished data. Tonnage reported as individual or as part of the combination products

In 2015, the sales of AGs made up 3.5% of the total sales for food-producing species (including horses), in mg/PCU, aggregated by 30 European countries. AGs are the sixth most commonly used antimicrobial class after tetracyclines, penicillins, sulfonamides, macrolides and polymyxins (Figure 1) (EMA/ESVAC, 2017).

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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 30 European countries, for 2015 (EMA/ESVAC, 2017).
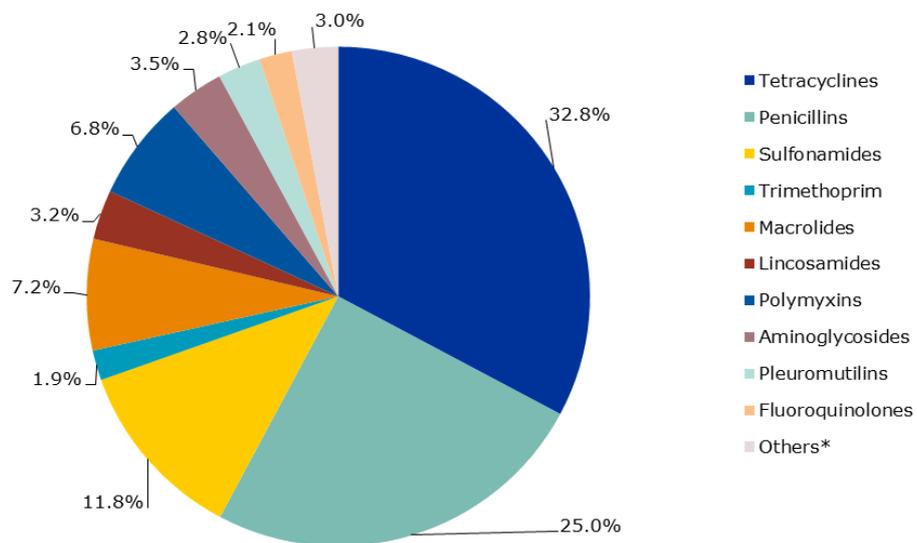
* Amphenicols, cephalosporins, other quinolones and other antibacterials (classified as such in the ATCvet system).

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.

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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 30 European countries for 2015 (EMA/ESVAC, 2017). Sales of spectinomycin and paromomycin are not included as they are reported under 'other antimicrobials' in the ESVAC report.
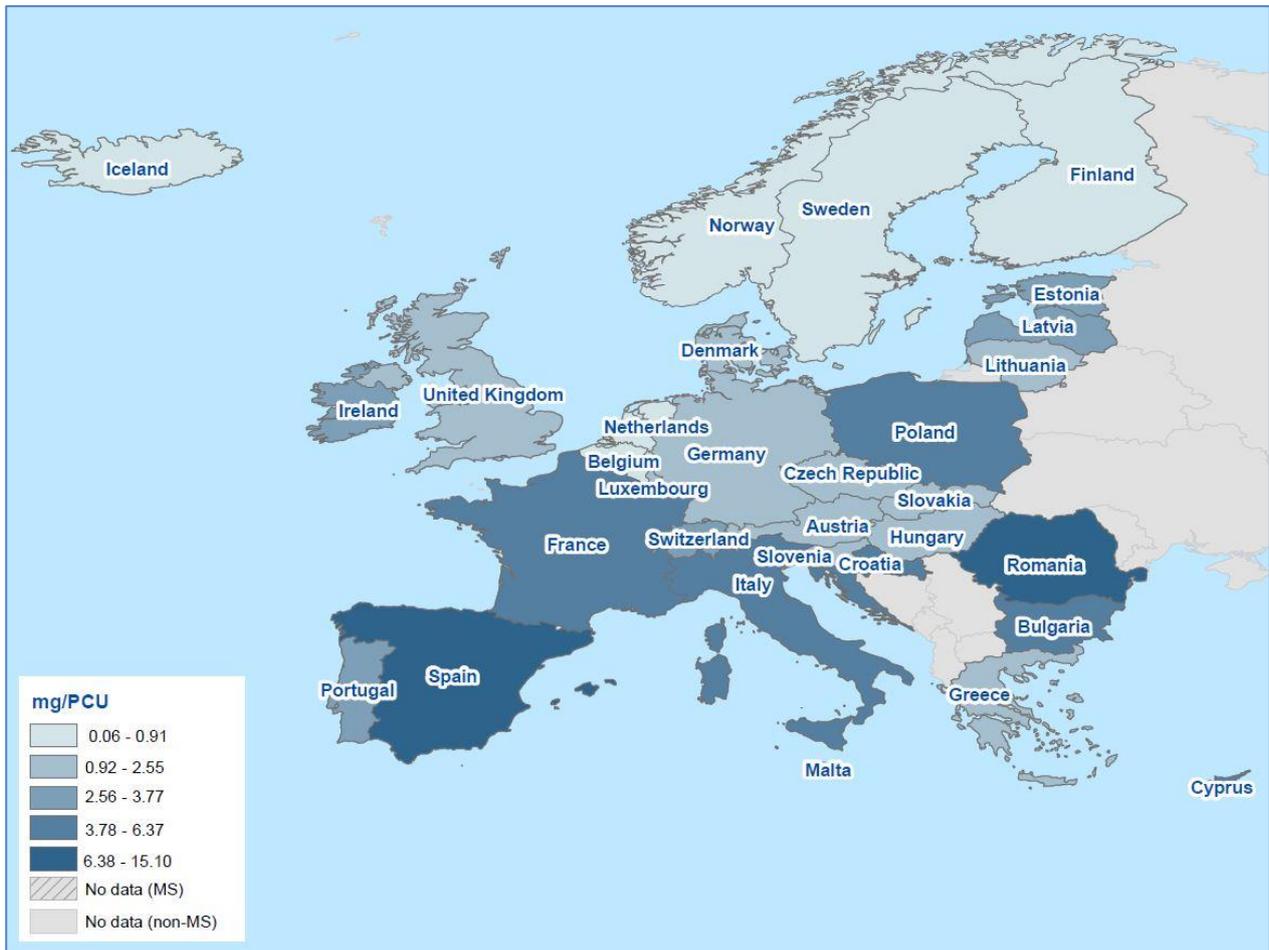
In the EU, approximately half of AG use is in oral formulations (premix, oral powder or soluble in drinking water) and about half is in injectable formulations (Figure 3 and Figure 4) (EMA/ESVAC, 2017). The most frequently used AGs are neomycin, dihydrostreptomycin and spectinomycin (Figure 5). Other substances from the group that are 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 this impacts on the withdrawal periods to be applied.

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Figure 3. Distribution of veterinary sales by pharmaceutical form for AGs, in mg/PCU, by country, for 2015 (EMA/ESVAC, 2017)
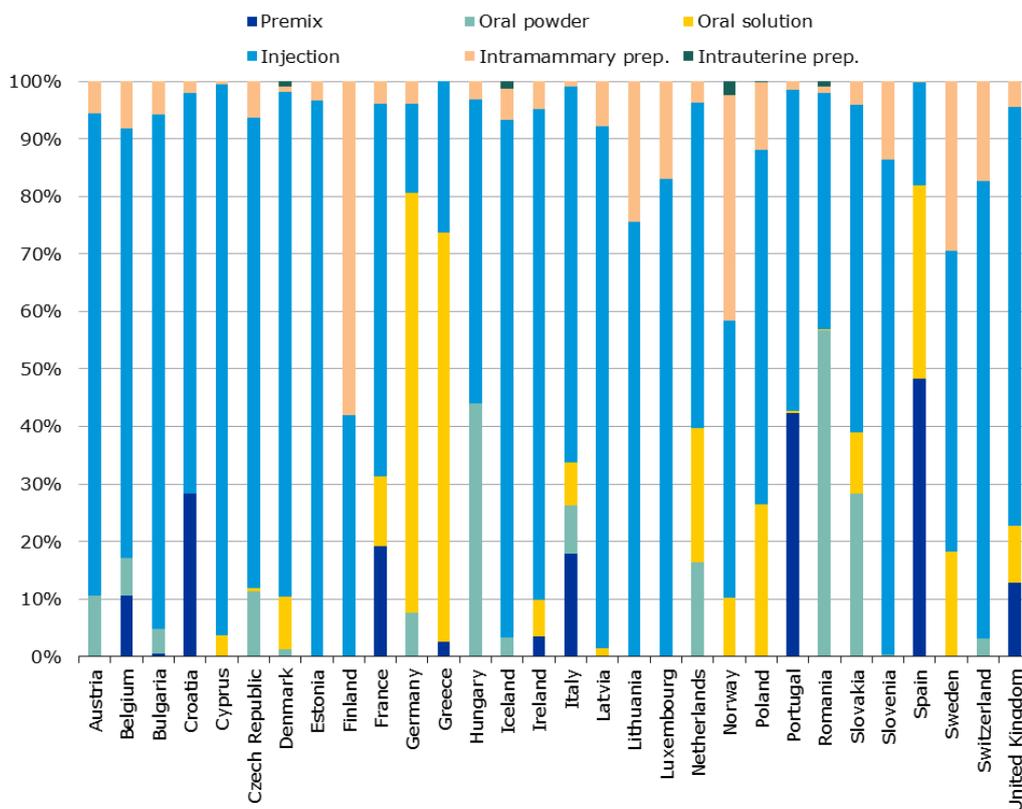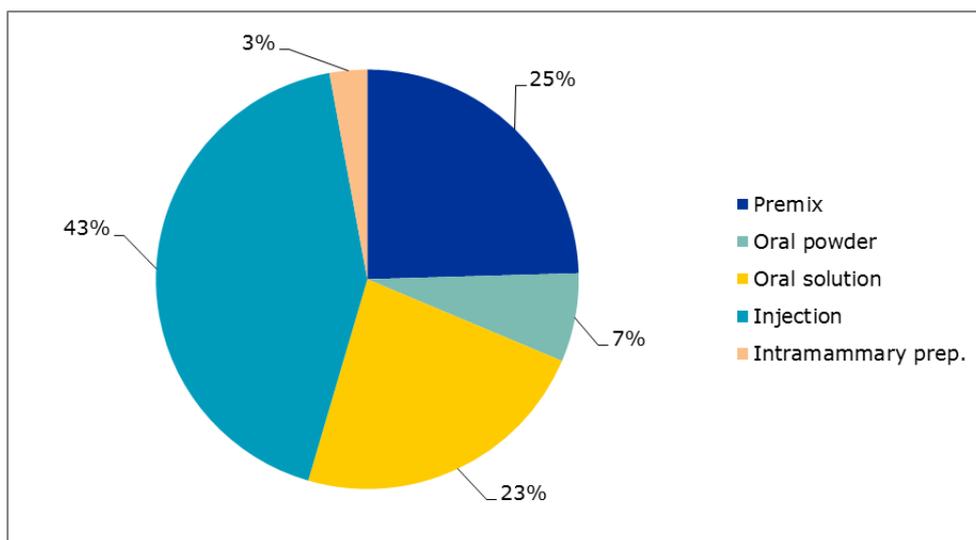

Figure 4. Distribution of veterinary sales by pharmaceutical form for AGs, for food-producing animals (including horses), in mg/PCU, aggregated by 30 European countries, for 2015 (ESVAC, as available in the Interactive Database). Sales of spectinomycin and paromomycin are not included.

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

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Figure 5. Sales of aminoglycosides, spectinomycin and paromomycin in food-producing species, in percentage of total mg/PCU, aggregated for 30 European countries in 2015 (ESVAC, unpublished data)
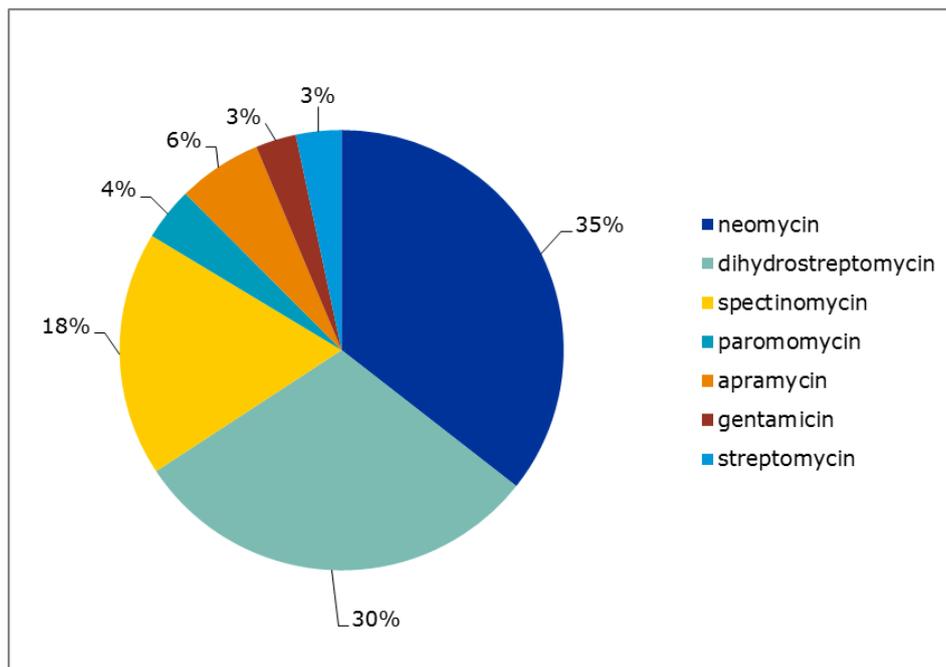
Minor sales (≤0.7%) of kanamycin and framycetin were also reported in 2015.

2.3. PK/PD relationship and dosing regimens

To date, no specific PK/PD targets (minimum value of a PK/PD index that is aimed to predict clinical efficacy) are established for AGs in veterinary medicine. Knowledge of the relationship between PK/PD indices (such as C_{max}/MIC, %T > MIC and AUC/MIC) and clinical outcomes for 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/MIC), 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 Cmax/MIC ratio as the PK/PD index of choice for AGs (gentamicin, tobramycin, amikacin). A Cmax/MIC ratio of 10 was best related to clinical outcome in 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 Cmax/MIC ratio of 10-12 was determined to minimize the survival and overgrowth of resistant strains (Toutain et al., 2002). If this preferable peak concentration to MIC ratio is obtained, most bacteria die within a short time and consequently the effect of the duration 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

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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, in order to reduce toxicity, to administer once-a-day to achieve a sufficiently low trough concentration, and to reduce treatment duration (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. The optimal doses and the ideal drug monitoring strategy are unknown for most target animal species. Dosages have to be modified in neonates and in animals with impaired liver or kidney function (Dowling, 2013).

In conclusion, prolonged treatment 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 for example tobramycin, gentamicin, amikacin and netilmicin are used systemically for treatment of (multidrug-resistant) Gram-negative infections such as those caused by *Pseudomonas* spp*.*, *Acinetobacter* spp., and Enterobacteriaceae. Kanamycin and amikacin are utilised for the treatment of multidrug-resistant tuberculosis (Labby and Garneau-Tsodikova, 2013); streptomycin was the first AG to be used against tuberculosis, but is nowadays rarely used. Amikacin may also be used against non-tuberculous mycobacterial infections. 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 without high-level resistance to AGs. 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. 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 (gentamicin, tobramycin, neomycin) are utilised for the treatment of ear and eye infections (Agence française de sécurité sanitaire des produits de santé, 2012; 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 cutaneous leishmaniasis. Spectinomycin is occasionally used for the treatment of gonorrhoea in patients allergic to penicillins (Table 2).

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

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intramuscular injection. Oral administration is limited to decontamination of the gut in intensive care units, as bioavailability following oral administration is low (Huttner et al., 2013).

In the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) survey, including data from 20 European countries, details on the consumption of individual AGs are not reported separately. Available ESAC-Net data from 2015 show that there are 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 of data from the initial European Surveillance of Antimicrobial Consumption project and describing outpatient parenteral antimicrobial 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 administered more 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
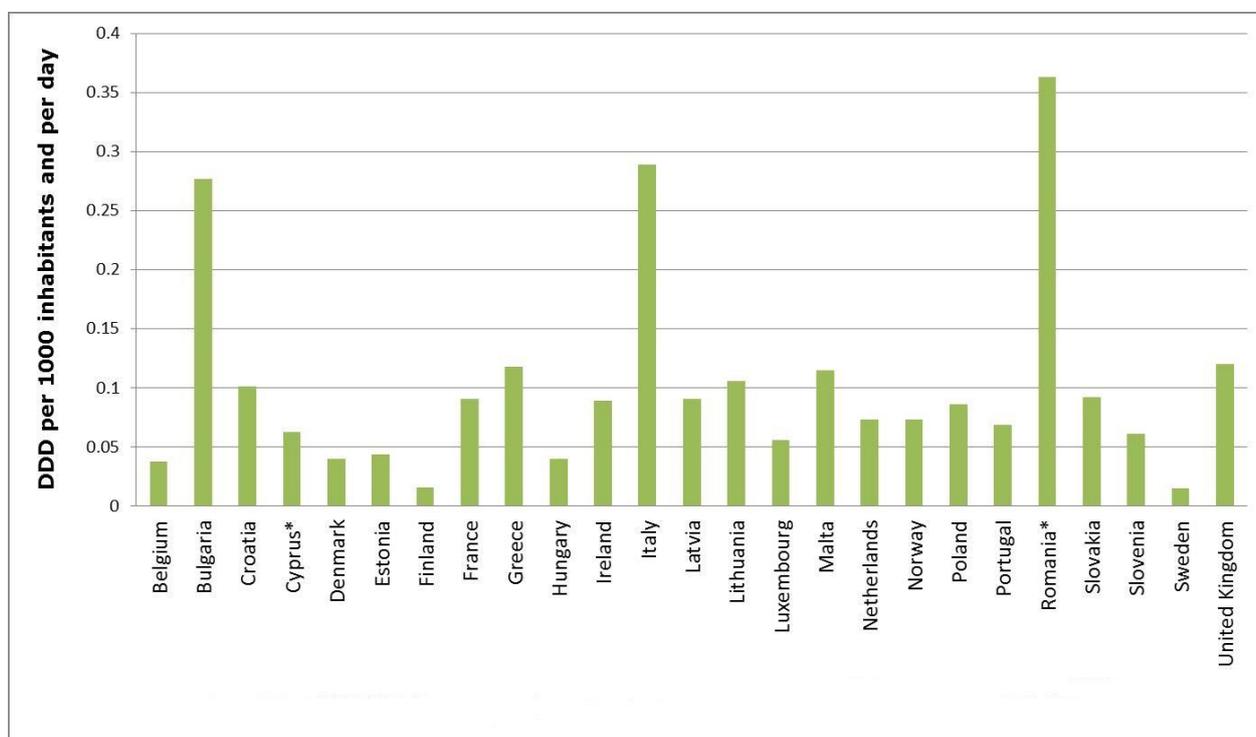
* Country provided only total care data.

Source: ESAC-Net (website, last accessed 2018)

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, 2017; Nethmap, 2017). Long-term monitoring in the Netherlands has shown an increase of the systemic use of AGs in Dutch hospitals (from 2.5 to 3.7 DDD/100 patient-days between 2006 and 2015) during the last decade. Despite a high overall antimicrobial consumption level in Belgian hospitals for 2016 (n=102, median value J01 577.1 DDDs/1000 patient days), for the subgroup of AGs a marked long term net decrease of 64.12% was found for the period 2003-2016

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(2016 median value, 6.74 DDD/1000 patient days) (Vandael; et al., submitted). In Norway and Denmark the use was stable (DANMAP, 2017; NORM/NORM-VET, 2014). Large teaching hospitals tend to have the highest use (Ingenbleek et al., 2015).

In addition to continuous surveillance as performed by the ESAC survey in outpatients and the hospital sector, targeted point prevalence surveys are done in hospitals (PPS HAI & AB) and long term care (HALT). The last published data show that on average 34.6% of patients receive antimicrobial therapy in acute care hospitals (ECDC, 2012) versus 4.4% in long term care facilities (LTCF) (HALT II) (ECDC, 2014a). In these settings, 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% (ECDC, 2012).

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	Used for MDR infections including tuberculosis	low Rarely used, generally not for first line treatment	*M. tuberculosis* is of limited zoonotic relevance, no plasmid-mediated transfer of resistance determinants in *M. tuberculosis*
gentamicin	Gram-negative infections, enterococcal and streptococcal endocarditis, brucellosis, tularaemia, plague, oral decolonisation, impregnated beads to prevent surgical site infections	high	Enterobacteriaceae – high risk of clonal and horizontal transfer of resistance genes;
amikacin	MDR Gram-negative infections, MDR tuberculosis, *Nocardia* spp. infections	high	Enterococci- risk of clonal (*E. faecalis*) and horizontal (*E. faecium* and *E. faecalis*) transfer of resistance genes
tobramycin	Gram-negative infections, Pseudomonas infections in cystic fibrosis	high	
(dihydro) streptomycin	MDR tuberculosis, but very rarely used	low	
spectinomycin	Gonorrhoea in patients allergic to penicillins	low	Gonorrhoea is not transmitted to humans from non-human sources transfer of resistance genes from non-human sources unlikely
paromomycin	Cryptosporidiosis	low	*C. parvum* is of zoonotic relevance
apramycin	No target	not used	selects for gentamicin

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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
			resistance in Enterobacteriaceae, such as *E. coli* and *Salmonella spp.*

4. Resistance mechanisms and propensity to spread from animals to humans

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 antimicrobial, 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, thereby 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 *Staphylococcus aureus* (Taber et al., 1987). Gentamicin resistance by inactivation of an outer-membrane porin, which serves as an entry for 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 baumannii*. 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), as well as 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. Therapeutic as well as sub-inhibitory AG concentrations can lead to resistance. The ability of bacteria to survive antimicrobial 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) provide an overview of most acquired resistance genes. A few novel spectinomycin resistance genes in staphylococci have subsequently been discovered (Jamrozy et al., 2014; Wendlandt et al., 2014; Wendlandt et al., 2013d). Resistance genes

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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 AAC enzymes are mainly found in Gram-negative bacteria such as Enterobacteriaceae, *Acinetobacter* spp. and *Pseudomonas* spp. They can 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., *Macrococcus* 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, and AAC(3)-IV to apramycin. 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 antimicrobial molecule. To date, there are five classes of ANTs categorised 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)- Ia -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 catalyse 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-positive organisms 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).

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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 antimicrobial action without interfering with other ribosomal functions. This mechanism was described mainly in different species of the AG-producing genera *Streptomyces* spp. and *Micromonospora* spp.. 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 ten additional genes encoding methylases have been reported: *rmtA, rmtB, rmtC, rmtD, rmtD2, rmtE*, *rmtF*, *rmtG* , *rmtH* and *npmA* (O'Hara et al., 2013; 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 antimicrobial classes, such as quinolones (Qnr proteins) or β-lactam antimicrobials (acquired AmpC-β-lactamases or extended-spectrum β-lactamases (ESBLs)). Recently these methyltransferases have been found in association with carbapenemases such as NDM-1 (Berçot et al., 2011; Hidalgo et al., 2013b; Ho et al., 2011). The genes (*rmtA, rmtB, rmtC, rmtD, rmtD2, rmtE, rmtF, rmtG, rmtH*) confer resistance to gentamicin, tobramycin, kanamycin and amikacin whereas *npmA* confers resistance to gentamicin, tobramycin, kanamycin, amikacin, neomycin and apramycin, but not to streptomycin or spectinomycin (Garneau-Tsodikova and Labby, 2016; Wachino and Arakawa, 2012).

Resistance to various AGs in staphylococci can be mediated by the genes *aac(6')-Ie-aph(2")-Ia* (kanamycin/gentamicin/tobramycin/amikacin resistance), *ant(4')-Ia* (kanamycin/neomycin/tobramycin resistance), *aph*(*3'*)-*III* (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 the *eis* gene 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

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resistance, suggesting that loss of function of this gene confers resistance (Cohen et al., 2014). To date, no plasmid-mediated resistance has been reported in *M. tuberculosis* or *M. bovis*.

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

Resistance gene/enzyme	Aminoglycoside to which this gene confers resistance
Acetlytranferases	
AAC (1)	neomycin, apramycin, paromomycin
AAC (2')	gentamicin, tobramycin, kanamycin, netilmicin, dibekacin
AAC (3) subclass I	gentamicin
AAC (3) subclass II	gentamicin, tobramycin, netilmicin, dibekacin, sisomycin, kanamycin
AAC (3) subclass III	gentamicin, tobramycin, netilmicin, neomycin
AAC (3) subclass IV	gentamicin, tobramycin, (kanamycin), netilmicin, neomycin, apramycin
AAC (6')	(amikacin), gentamicin
Phosphotransferases	
APH (2'')	gentamicin
APH (2'')/ AAC (6')	gentamicin, tobramycin, kanamycin, (amikacin)
APH (3') subclass I	kanamycin, neomycin, paromomycin
APH (3') subclass II	kanamycin, neomycin, paromomycin
APH (3') subclass III	kanamycin, neomycin, paromomycin, (amikacin)
APH (3'')	streptomycin
APH (6)	streptomycin
APH (9)	spectinomycin
Nucleotyltransferases	
ANT (2')	gentamicin, tobramycin, kanamycin, dibekacin, sisomycin
ANT (3'')	streptomycin, spectinomycin
ANT (4')	tobramycin, amikacin, isepamicin (dibekacin)
ANT (6)	streptomycin
ANT (9)	spectinomycin
Methyltransferases	
ArmA	gentamicin, tobramycin, kanamycin, amikacin
RmtA, RmtB, RmtC, RmtD, RmtD2, RmtE, Rmt, RmtG, RmtH	gentamicin, tobramycin, kanamycin, amikacin
NpmA	gentamicin, tobramycin, kanamycin, amikacin, neomycin, apramycin

5. Consideration on susceptibility testing of aminoglycosides

Susceptibility data from national monitoring programmes are available and MIC determination via broth microdilution is the most frequently used method in these programmes. 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

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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). Alternatively, 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).

Based on the high occurrence 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.

To date, EUCAST has no veterinary-specific breakpoints. In contrast 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).

Misleading results can also be obtained when testing *Salmonella* spp. for AG susceptibility as they might be reported as susceptible *in vitro*, while they are ineffective therapeutically due to the intracellular location of the microorganisms and the difficulty for AGs to cross the eukaryotic membrane. In addition, the low pH within vacuoles of the phagosome can limit the efficacy of AGs *in vivo* (CLSI, 2013; CLSI, 2015b).

A recent study showed that results of susceptibility testing for gentamicin for *K. pneumoniae* resistant to carbapenems obtained with Vitek 2 and E-test 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* spp. isolates against tobramycin using MALDI-TOF MS technology has been explored and it 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).

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6. Occurrence of resistance in bacteria from animals and humans

6.1. Food-producing animals

In general, in food-producing animals, resistance to streptomycin is very common while resistance to the other AGs is detected less frequently. In the EU monitoring programs (MARAN, DANMAP, EFSA), ECOFFS are used for the interpretation of antimicrobial susceptibility testing in food-producing animals and therefore isolates reported as "resistant" in this paragraph are not always clinically resistant. In the Netherlands, resistance in *Salmonella* spp. isolates was uncommon for gentamicin and kanamycin (2-3%), but 31% of the isolates were resistant to streptomycin. In *Campylobacter* spp. 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%). In Denmark, porcine *Salmonella* spp. 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* spp. 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 in *E. coli*, enterococci, *Campylobacte*r spp. and *Salmonella* spp. isolates were low in 2014 and 2015 (DANMAP, 2016).

Data from 17 MS show that resistance to gentamicin in *Salmonella* spp. 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* spp. isolates from turkeys 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* spp. 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).

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Equine *E. coli* isolates were generally susceptible to gentamicin and the resistance rate was only 8.8% in Germany (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%) in the UK (Johns and Adams, 2015).

Characterization of 227 *Streptococcus suis* isolates 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 sulfonamide 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 16S rRNA 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, chickens, 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 Tn*5405*-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), in France, the percentages of feline *E. coli* susceptible 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

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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 [*aac(6')-Ie-aph(2')-Ia*] (88.3%), kanamycin [*aph(3')-III*] (90.3%), streptomycin [*ant(6)-Ia*] (90.3%), streptothricin (*sat4*) (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* sequence type (ST) 11 isolates from dogs and cats were found to be resistant to AGs, and the *armA* gene 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 $bla_{CTX-M-9}$ group genes within the same incompatibility group (Inc) FII plasmid (Deng et al., 2011).

6.3. Humans

Data from the ECDC EARS-Net surveillance indicate that resistance of *Klebsiella pneumoniae* isolates to AGs was below 5% in Scandinavian countries and Austria, between 5-10% in the Netherlands, Germany and the UK, between 25-50% in France, Italy, Portugal, Lithuania, Hungary, Croatia and Czech Republic, while it was between 50% and 75% in Greece, Bulgaria, Romania, Poland and Slovakia. For *E.coli*, AG resistance was below 5% in Finland and Iceland, between 5-10% in Sweden, Norway, the Netherlands, Germany, France, Belgium, the UK and Austria (among others), between 10-25% in Spain, Italy, Ireland, Greece and many eastern European countries and 35% in Bulgaria. High level gentamicin resistance in *Enterococcus faecalis* was between 10 and 25% in Sweden, Norway, France, Belgium, the Netherlands, Iceland and Greece; it was between 25-50% in most other EU MS, while a prevalence of 56% was found in Romania. Even higher prevalences of high level gentamicin resistance were reported for *Enterococcus faecium* with levels above 70% in the Netherlands, Czech Republic, Romania, Lithuania and Bulgaria (ECDC, website, last accessed 2018). Generally countries with the highest human consumption of AGs, such as Bulgaria and Romania, also reported the highest levels of AG resistance.

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 in 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-encoding 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-

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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 substance 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). Gentamicin resistance increased in clinical *E. coli* isolates from broiler chickens in Québec, despite the fact that this antimicrobial was no longer used. This increase coincided with the use of a spectinomycin-lincomycin combination for prevention of colibacillosis, including *in-ovo* injection. The major genes identified for resistance to gentamicin and spectinomycin were *aac(3)-VI* and *aadA*, respectively. The *aadA* and *aac(3)-VI* genes were located on a modified class 1 integron. Therefore the use of spectinomycin-lincomycin resulted not only in an increase in spectinomycin resistance, but also co-selected for gentamicin resistance (Chalmers et al., 2017).

The use of various antimicrobials, including kanamycin, at concentrations *in vitro* far below the MIC, promoted the selection of an ESBL-conferring plasmid conferring resistance not only to β-lactams but also to AGs, tetracycline, trimethoprim, sulfonamides, and erythromycin, as well as biocides and heavy metals (Gullberg et al., 2014). These findings suggest that low concentrations of antimicrobials 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 antimicrobial-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 to AGs and other antimicrobial classes 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 antimicrobial 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 and are widely used for the treatment of common infections in food-producing species and companion animals. They are categorised as veterinary critically important antimicrobials (VCIAs) by the OIE based on the wide range and nature of the diseases they are used to treat in animals and the limited availability of economic alternatives for some infections. 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 the EU. In horses, for example, gentamicin is one of the few available antimicrobials for treating Gram-negative infections, the alternative treatment options being trimethoprim/sulphonamide combinations (TMPS),

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3rd- and 4th-generation cephalosporins and fluoroquinolones. The latter two antimicrobials, however, are also to be used restrictively and resistance to TMPS is very common among Gram-negative bacteria. In pigs, AGs are important drugs for the treatment of post-weaning diarrhoea. Alternatives include 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, but these antimicrobials are included in AMEG category 2. With regard to infections with *Pseudomonas*, 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 topical applications, alternatives include polymyxins and fluoroquinolones, which are included in AMEG category 2. For systemic treatment of *Pseudomonas* infections, fluoroquinolones are one of the few other treatment options besides AGs and the use of this class of antimicrobials should be restricted to conditions where no alternative treatment options are available.

In conclusion, AGs are very important for treatment of a broad range of Gram-negative infections in animals. Although there are effective alternatives if resistance develops, these are frequently substances included in AMEG's category 2 (higher risk to human health), hence having restrictions on their use in animals.

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 antimicrobial 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), but these compounds are not on the market as yet.

To date, 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 *A. baumannii* and Enterobacteriaceae, including *Salmonella* spp., that co-produce 16S-rRNA methylases and carbapenemases 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, the resistance genes *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. It should be noted that a systematic review assessed mortality, treatment failures and

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antimicrobial resistance by comparing β-lactam therapy versus any combination of a β-lactam with an AG for human cases of blood stream infections. The authors concluded that the addition of an AG to β-lactams for treatment of sepsis should be discouraged, since mortality rates were not improved and the addition of AGs considerably increased the risk for nephrotoxicity. The subgroup of *Pseudomonas aeruginosa* infections was underpowered to examine effects (Paul et al., 2014).

Besides infections with multidrug-resistant Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp., multidrug-resistant tuberculosis and Gram-positive endocarditis are among the diseases for which availability of AGs is critically important due to few alternatives (EMA/AMEG, 2014). For enterococcal endocarditis caused by enterococci without high-level AG resistance, ampicillin combined with gentamicin is considered the regimen of first choice. During the last decade alternative treatment options including high dose daptomycin and the combination of ampicillin with ceftriaxone have been explored and have been shown to be equally effective in certain studies (Carugati et al., 2013; Falcone et al., 2015; Fernández-Hidalgo et al., 2013).

In conclusion, AGs are important drugs for the treatment of infections with multidrug-resistant Gram-negative bacteria, enterococcal endocarditis 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's answers to the request for scientific advice on the impact on public health and animal health of the use of antimicrobials 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 and 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. Antimicrobial resistance in several *Salmonella enterica* serovars is due to genomic islands carrying a class 1 integron, which carries the relevant resistance genes. *Salmonella* genomic island 1 (SGI1) was found in *Salmonella enterica* serovar Typhimurium Definitive phage type (DT) 104 (=S. Typhimurium DT 104) isolates, which are resistant to ampicillin, chloramphenicol, florfenicol, streptomycin, spectinomycin, sulfonamides and tetracyclines. Several Salmonella serovars have since been shown to harbour SGI1 or related islands. SGI1 is an integrative mobilisable 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.

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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 be transmitted between animals and humans. AGs are used for treatment of zoonotic infections such as tuberculosis, brucellosis and tularaemia. Even bacteria causing human infections not directly linked to animals may acquire resistance determinants from bacteria with zoonotic potential. The indirect risk from the use of AGs in food-producing animals should therefore be taken into account in determining risk profiles. 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-beta-lactamase 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 Tn*501*-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).

Combined apramycin and hygromycin B resistance is mediated by the *aac*(3)IV and *hph*B genes. These genes are part of one resistance gene operon associated with an insertion sequence IS*140*, which is found on plasmids. The organisation of *aac(3)IV* and *hph*B is such that they are always co-transferred. Apramycin and hygromycin B are used exclusively in animals. The gene *aac(3)IV*, conferring cross-resistance to gentamicin was first identified in *E. coli* and *Salmonella* Typhimurium from animals in France and the United Kingdom (Chaslus-Dancla et al., 1986; Wray et al., 1986). A high degree of genetic homology between plasmids harbouring *aac*(3)IV and *hph*B of human and animal origin has been demonstrated (Chaslus-Dancla et al., 1991; Salauze et al., 1990). In a prospective study on a pig farm, Hunter et al. (1994) demonstrated a widespread dissemination of plasmids harbouring *aac(3)IV* in *E. coli* from pigs, calves, the farmer and the environment. *Klebsiella pneumoniae* with a slightly smaller conjugative plasmid and similar resistance pattern was isolated from the farmer's wife, indicative of animal-to-human transmission of resistance genes.

Extended-spectrum or plasmidic AmpC β-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 has been 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 of 267 isolates, most of which were clonally unrelated. The coexistence of the *rmtB* gene with the $bla_{CTX-M-9}$ 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 to date the main resistance mechanism for mycobacteria is chromosomal mutation. In addition, tuberculosis in humans is mostly caused by *M. tuberculosis*, which is mainly 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: 2018).

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Molecular epidemiological studies based on multi-locus sequence typing (MLST) have 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-acquired, 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). Although *E. faecium* isolates from animals do not seem to be a direct hazard to human health by clonal transmission, they could act as donors of resistance genes to pathogenic enterococci through horizontal gene transfer. For *E. faecalis* 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).

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

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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

[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 mobilisation described; 2, gene is exclusively on the core bacterial chromosome; 3, gene is on a mobile genetic element, e.g. plasmid.

[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, 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 chromosome in a particular successful resistant clone; 3, gene is on a mobile genetic element, e.g. plasmid, in a particular successful resistant clone.

[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-mobilisation of the gene or risk factor described; 2, gene is either co-mobilised or a risk factor has been described; 3, gene is co-mobilised and a risk factor has been described.

[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 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, 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.

[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 resistance mobile genetic element detected in bacterial isolates of animal and human origin; Resistant bacteria - Defined as a similar bacterium harbouring a resistance gene (either chromosomally or mobile genetic element-encoded) of 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 animals and humans; 4, genes and mobile genetic elements and resistant bacteria similar between animals and humans.

* Based on surveillance data from food-borne pathogenic and commensal bacteria (EFSA/ECDC, 2017)

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11. Discussion

AGs are bactericidal antimicrobials 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 mg/PCU) for food-producing species in 30 European countries in 2015. The amount of AGs used in animals varies significantly between European countries and the reasons for this are not known. The most commonly sold AGs were neomycin, dihydrostreptomycin and spectinomycin: together they accounted for 83% of the total sales of AGs, while sales of gentamicin account for only 3%.

In human medicine, AGs are used primarily in infections involving Gram-negative bacteria, such as *Pseudomonas* spp*., Acinetobacter* spp. and Enterobacteriaceae*,* in combination with beta-lactams or vancomycin for the treatment of endocarditis caused by enterococci or streptococci and for treatment of multidrug-resistant tuberculosis. 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.

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 1) (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 1 report).

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 three-five days. Some products are licensed for more than seven days, some for in-feed use even for 21 or 28 days. The need for long treatment durations and parenteral administrations more than once daily should be reviewed. Any indications for treatment of salmonella infections in chicken should be in line with EC regulations and take account of the public health risk. Indications for therapy by oral administration for systemic diseases should also be reviewed.

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

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. Such 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 such as streptomycin or spectinomycin are generally different from those of

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gentamicin or tobramycin and other AGs. With some exceptions, resistance to streptomycin and spectinomycin is generally common in isolates from animals, including those with zoonotic potential, whilst resistance to gentamicin, amikacin and kanamycin is 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 food-borne 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 generally not be treated with AGs. *E. coli* and enterococci, 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* spp. to *Klebsiella* spp. 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 of the emergence of AG resistance and gene transfer resulting from the use of oral products, which are indicated mostly to treat enteric infections in pigs, chickens and calves (apramycin, neomycin, streptomycin, spectinomycin, gentamicin), is much higher than for other formulations as the former 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* spp., but fortunately the percentage of resistance to gentamicin in these bacteria is relatively low, most likely due to differences in the resistance mechanisms and differences in the amounts of such antimicrobials used in veterinary medicine.

The risk for resistant infections in humans resulting from the use of topical products including drops used to treat eye and ear infections (mainly infections with *Pseudomonas* spp.) in companion animals is generally regarded as low, as treatment involves individual animals and this local route of administration does not result in a 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 bacteria from humans from the use of AGs (streptomycin, gentamicin) as injectables will generally be lower if animals are treated individually rather than as a group.

In veterinary medicine, AGs are one of the few treatment options for infections with *Pseudomonas* spp. in different animal species, for infections with Gram-negative bacteria in horses and for treatment of post-weaning diarrhoea caused by *E. coli* in pigs. Although effective alternatives are available, these are frequently substances included in AMEG's category 2.

In human medicine, AGs are important for the treatment of infections with *Pseudomonas* spp., *Acinetobacter* spp., multidrug-resistant Enterobacteriaceae, enterococci and multidrug-resistant tuberculosis; they are, however, rarely the sole treatment option. The risk of transmission of resistant Enterobacteriaceae to humans from non-human sources is regarded as high. AGs have been

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considered critical for humans as a sole or one of limited treatment options for enterococcal endocarditis. For enterococcal endocarditis and bacteraemia alternative treatment options are now available and there are studies indicating that combination therapy with two beta-lactams is as effective as combination therapy with AGs, with less toxicity for patients (Carugati et al., 2013; Fernández-Hidalgo et al., 2013; Leone et al., 2016; Paul et al., 2014; Pericas et al., 2014).

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. For *E. coli*, *Salmonella* spp. 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 in humans. 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 of multidrug resistant tuberculosis are caused by *M. tuberculosis,* which is mainly transmitted from humans-to-humans. Bovine tuberculosis, caused by *M. bovis*, is rare in Europe overall and the emergence of multidrug resistant *M. bovis* strains is regarded unlikely as animals infected with tuberculosis are not treated but euthanized.

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 and which have been categorized in AMEG category 2 (Pardon et al., 2017). The consequences of the use of these alternatives instead of AGs should also be taken into account. 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 both in human and veterinary medicine is of great importance.

12. Conclusion

- The Antimicrobial Advice *ad hoc* Expert Group (AMEG) categorisation considers the risk to public health from AMR due to the use of antimicrobials in veterinary medicine. Considering the AMEG criteria, all veterinary-authorised AGs, including streptomycin, would be placed in Category 2 given (i) their importance in human medicine, (ii) the high potential for transmission of resistance determinants between animals and humans, and (iii) the potential for co-selection of resistance as described by the AMEG. According to the opinion of CVMP, use of AGs in veterinary medicine has a lower risk to human health compared to use of fluoroquinolones and 3rd- and 4th-generation cephalosporins as AGs are used for a lower absolute number of human patients affected by all diseases for which these antimicrobials are one of few therapies available and are used less often for other types of infections than 3rd- and 4th-generation cephalosporins and fluoroquinolones in human medicine (WHO). These considerations could support a further stratification of the AMEG's categorization, as is currently being undertaken by the AMEG group.

- 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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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 2015, AGs accounted for 3.5% of the total sales of veterinary antimicrobials in mg/PCU in 30 European countries. 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. The genes encoding resistance to AGs such as streptomycin or spectinomycin are generally different from those of gentamicin or tobramycin and other AGs and there is usually no complete cross-resistance between antimicrobials in this class; however, there is evidence that use of apramycin in pigs can select for gentamicin-resistant Enterobacteriaceae, including *E. coli* and *Salmonella* spp. Amongst animal pathogens, high levels of resistance have been reported 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, 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. Enterobacteriaceae, LA-MRSA and *Enterococcus* 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 human medicine, the most used AGs were gentamicin, amikacin and tobramycin. 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 for treatment of enterococcal endocarditis, multidrug-resistant Gram-negative bacteria (especially Enterobacteriaceae and *Pseudomonas* spp.) and multidrug-resistant tuberculosis.

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Recommendations

Proposal on categorisation for consideration by AMEG

- The Antimicrobial Advice *ad hoc* Expert Group (AMEG) categorisation considers the risk to public health from AMR due to the use of antimicrobials in veterinary medicine. Considering the current AMEG categorization criteria, all veterinary-authorised AGs, including streptomycin, would be placed in Category 2 (higher risk for public health) given (i) their importance in human medicine, (ii) the high potential for transmission of resistance determinants between animals and humans, and (iii) the potential for co-selection of resistance as described by the AMEG. According to the opinion of CVMP, use of AGs in veterinary medicine has a lower risk to human health compared to use of fluoroquinolones and 3rd- and 4th-generation cephalosporins as AGs are used for a lower absolute number of human patients affected by all diseases for which these antimicrobials are one of few therapies available and are used less often for other types of infections than 3rd- and 4th-generation cephalosporins and fluoroquinolones in human medicine (WHO). These considerations could support a further stratification of the AMEG's categorization, as is currently being undertaken by the AMEG group.

- 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 (dihydro)streptomycin as sole active substance or fixed combinations of AGs or AGs 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 rationale for indications for treatment of systemic diseases with aminoglycosides that have very low oral bioavailability should be reviewed.

- The need for prolonged treatment duration for certain products administered orally to groups of animals should be reviewed in the context of the specific indications.

- In reference to the above three recommendations and the scope of any referral procedures, review of groups of products would be prioritised according to relative risk.

- Based on the high occurrence 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, as they are predictive of clinical efficacy and enable optimisation of dosing regimens for AGs that are administered parenterally.

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- Susceptibility testing should be standardised and veterinary clinical breakpoints should be established for AGs in order to enable 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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