21 June 2018
EMA/CVMP/AWP/726389/2017
Veterinary Medicines Division

Overview of comments received on 'Reflection paper on use of aminoglycosides in animals in the European Union: development of resistance and impact on human and animal health' (EMA/CVMP/AWP/721118/2014)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1.	Swiss Agency for Therapeutic Products (Swissmedic)
2.	Ceva santé Animale
3.	Federation of Veterinarians of Europe (FVE)
4.	AnimalhealthEurope

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1. General comments – overview

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1.	The reflection paper gives a good general overview on the aminoglycoside use and resistance mechanisms in bacteria from animals and humans. Swissmedic shares the opinion that aminoglycosides do not have the same risk profile as fluoroquinolones and 3rd- and 4th-generation cephalosporins and supports a further stratification of the categorisation. There are some suggestions that might improve the quality of this reflection paper: 1) The use of several nomenclatures throughout the manuscript may lead to confusion. It is important to use one single nomenclature due to the number of different resistance mechanisms for aminoglycosides. Please see specific comments for proposed changes. 2) It should be directly stated that the monitoring programs of resistance in bacteria from animals in Europe do not permit the detection of aminoglycoside methylases since MIC of amikacin is generally not measured. 3) The association of aminoglycoside methylases with carbapenemases should be more emphasized. This association is increasingly reported in bacteria from human origin.	1.1. Thank you for your comment. Nomenclature was checked and corrected. 1.2. It is correct that MIC's of amikacin are not included in EU monitoring programs. Gentamicin is, however, included and also predicts the presence of methylases. 1.3 We believe that the methylases and their association with carbapenemases are already mentioned in the manuscript and therefore there is no need to emphasize it more.
2.	The aminoglycosides (AGs) were some of the first antibiotics used in veterinary medicine and have been in use since the 1950s. Their chemical structure varies greatly; to such an extent that the breakpoint values defined are often molecule-dependent (Vet01-S2, 2013; CA-SFM, 2016). It is important to consider and to compare the information available on the mechanisms of resistance responsible for resistance emergence towards the old and recent AG molecules and consequently the impact of no-cross-resistance between old AG and the newer generation ones in the current context of the categorisation of the AG antibiotics. There are a number of different generations of AGs. Streptomycin belongs to the first generation (Hancock R.E.W., 1981) and resistance to streptomycin does not compromise a subsequent usage of latest generations AG. Amikacin is a more recent AG used commonly in human medicine, therefore the emergence of resistance to this latter would impair its clinical	2. It is already mentioned that the resistance mechanisms and breakpoints vary between different AG molecules. This relates more to their chemical structure than to whether they are old or new. It is mentioned in the RP that the resistance mechanism for streptomycin and spectinomycin differ from that of the other AGs.

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	efficacy. It is therefore crucial to take into account the fact that the AGs used in veterinary medicine (Particularly the first generations) do not generate cross-resistance to the AGs commonly used in human medicine, such as amikacin. This scientific information should allow the establishment of a comprehensive ranking of the AGs used in veterinary medicine, which would recommend a preferential usage of old AGs as first line treatment in veterinary medicine. In the contrary, AGs whose resistance overlaps to a greater degree should be rightly ranked in categories that are more critical. The nature of the genetic supports involved in resistance, primarily involving numerous plasmid-mediated, AG-modifying enzymes, is widely known (Vakulenko S.B. et al, 2003). When screening the antibiotic profiles generated through these enzymes, it is interesting to note that, unlike the other AG drugs, streptomycin only induces the expression of a limited series of enzymes in the bacterium (AG phosphotransferases, type APH(3")-Ia, Ib and APH(6)-Ia, Ib, Ic and Id encoded by the strA-strB genes (Sunde M. et al, 2005); and no AG acetyl transferase (enzymes very commonly expressed in the inducible resistance towards more recent AGs); AG nucleotidyltransferases, types ANT(3')-I and ANT(6')-I encoded by the aadA gene (Sunde M. et al, 2005). The article published by Vakulenko S.B. et al, 2003 raises a very interesting point since it clearly demonstrates that the potential for cross-resistance between streptomycin and the other AGs is very low since the majority of the inducible enzymes recognise streptomycin as it's rightly highlighted in the reflection paper. Another mode of bacterial resistance to AGs involves the genetic modification of ribosomal receptor sites to prevent the binding of the antibiotic and induce misreading as well as inhibiting protein synthesis. Small differences in the structure of the AG, and particularly the presence and position of 2'-amino groups, can result in substantial differences in the inhibition of protein synthesis. For example: the molecular structure of streptomycin differ from that of kanamycin or amikacin and the bacterial resistance, which results from ribosomal modification and inhibited binding capability, is also different (Benveniste R. et al, 1973). The induction of this type of resistance to streptomycin does not confer resistance to more recent generations of AGs (gentamycin, kanamycin, amikacin). This point is in accordance with the reflection	Most AGs used in veterinary medicine are also used in human medicine. Many AGs used in veterinary medicine can generate cross-resistance. Streptomycin is the exception. Criteria for the categorisation of antimicrobials and the need for further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG). This is beyond the scope of this reflection paper. We are aware of the fact that there is limited cross-resistance between streptomycin and the other AGs. This is clearly stated in the reflection paper.

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	paper (page 22 line 576). Indeed, it is well known that amikacin binds strongly to the 50S ribosomal sub-unit, unlike streptomycin, which binds with varying degrees of affinity to the 30S ribosomal sub-unit. Furthermore, gentamycin shares some common binding sites with amikacin on the 50S ribosomal sub-unit, which is not the case for streptomycin (Bryan L.E. et al, 1983). Moreover, from the epidemiological point of view and about the widespread of plasmid mediated ESBL, the main AG resistance determinants found on the clinically significant and widely spread epidemic plasmids contain resistance determinants mainly of the latest generation AG molecules and those used in human medicine such as gentamycin, kanamycin, tobramycin and amikacin in addition to resistance determinants of third generation cephalosporins and fluoroquinolones (Jin W. et al, 2015; Doi Y.et al, 2004; Cortes G. et al, 2017) One combination has stood the test of time. It is benzylpenicillin with dihydrostreptomycin (DHS). This drug used since as far back as the 1950s, when Prof. Jawets was publishing articles (Jawets E. et al, 1950), is composed of a combination of two narrow-spectrum molecules, and is currently still widely used in veterinary medicine, particularly as a parenteral administration and at all stages of animal rearing. Penicillin (belonging to the first category of the antibiotic in the EMA list) synergises the penetration of streptomycin into the bacteria's cytoplasm, with this activity persisting even if the microorganism develops ribosomal resistance. Consequently, the afflux of streptomycin into the cytoplasm may ultimately overcome the ribosomal resistance mechanisms put in place, so long as the levels of resistance generated are not exceptionally high. For example, Yee Y. et al, 1986 demonstrated that penicillin also promoted the uptake of streptomycin in ribosomal-resistant organisms such as Enterococcus. Although resistance frequency to streptomycin alone is relatively high (30-50%), the synergistic effect of the combination of dehydrostreptomycin and penicillin on streptomycin resistant microorganisms correlates with the results obtained by Schwarz S. et al, 2007; Plotz P.H. et al, 1962 since strains which have MIC with regard to dihydrostreptomycin of > 128	Streptomycin resistance is very common in commensals as well as clinical isolates. Resistance determinants mediating resistance to streptomycin are very common, for example in in integrons. Bifunctional aminoglycoside modifying enzyme gene *aac(6')-Ie-aph(2'')-Ia* is the

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	µg/ml are sensitive to combinations exhibiting much lower concentrations of benzyl-penicillin and DHS. Another more recent study published at the European Congress of Chemotherapy and Infectious Diseases (ECCMID) demonstrated the efficacy of benzylpenicillin/DHS on streptomycin resistant strains and more importantly the absence of cross-resistance between streptomycin resistant strains and the newer generation AG (M'Zali F. et al, 2016). In addition, the frequency of resistance emergence to benzylpenicillin/DHS is much lower when compared to the frequency of resistance to streptomycin alone. Regardless to the absence of cross-resistance profiles with newer AGs (Vakulenko S.B. et al, 2003) and in accordance with both tables 3 page 607 and table 2 page 481, it is important to preserve the use of the old AG molecules such as streptomycin and spectinomycin as first line therapies, and as resistance frequencies are high antimicrobial susceptibility profiles of the micro-organisms should be recommended. In addition the risk of resistance emergence is greater when the usage of this drugs is through the oral form or group therapy so we recommend the parenteral administration of these drugs as the main route to preserve their activity and diminish the risk of resistance emergence. It is important as well to preserve benzylpenicillin/DHS combination as a first-line parenteral therapy, since (a) This old yet effective combination is exclusively used in veterinary medicine and is still efficient (M'Zali F. et al, 2016); (b) The drugs have no impact on the commensal intestinal coliforms (since they are naturally resistant to benzylpenicillin, and dihydrostreptomycin is entirely eliminated via the renal route); (c) It generates a synergistic effect on the microorganisms without generating cross-resistance to the others, more recent, aminoglycoside drugs (no crossed effects with deoxystreptamines: gentamicin and kanamycin); (D) Dihydrostreptomycin does not play a major role in the spread of genetic determinants encoding for ESBLs as resistance genes are mainly located on chromosomes (which is not the case for the other AGs);	predominant gene responsible for high-level AG resistance in enterococci. Not Agreed. There is no evidence of synergy between penicillin and streptomycin (at current doses and for current indications) *in vivo*. There is no clinical information that supports that the penicillin-streptomycin combination offers any advantage compared to penicillin alone for the main indications. Staphylococci (when penicillin-sensitive), and streptococci, already have low penicillin-MICs. When staphylococci produce penicillinase there will be no synergy at all. For Pasteurella ssp. there is lack of synergy even in vitro (see Schwartz) and E, coli has too high MICs for synergy for any clinical effect at authorised doses. The penicillin/streptomycin combination was withdrawn from the US market in 1993 as no evidence of clinical synergy was presented. a. Both penicillin and streptomycin are used in human medicine, although not in combination. b. There is impact on the intestinal flora as many

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	(E) Penicillin is already ranked in the first category of EMA list and it is an excellent opportunity to maintain in the first category such old association as it is for TMP/sulfa with very limited efficacy. Proposed category change: Maintain in Category 1 old AGs such as (dihydro) streptomycin which is very rarely used in human medicine compared to more recent AG generations (see pages 415, 430 -431 and page 441-432 of the reflection paper) and rank in Category 1 Benzypenicillin/(dihydro)streptomycin combination, especially for parenteral applications. In addition it is important to recommend that antibiotics susceptibility profiles of the micro-organisms are warranted in order to achieve an optimal outcome. References: - Benveniste R., Davies J. Structure-activity relationships among the aminoglycoside antibiotics. Role of hydroxyl and amino groups. Journal of Antimicrobial Agents and Chemotherapy. 1973 ; 4 : 402. - Brilene T., Soeorg H., Kiis M., Sepp E., Koljalg S., Loivukene K., Jürna-Ellam M., Kalinina J., Stsepetova J., Metsvaht T., Lutsar I. In vitro synergy of oxacillin and gentamicin against coagulase-negative staphylococci from blood cultures of neonates with late-onset sepsis. APMIS. 2013 Sep;121(9):859-64. - Bryan L.E., Kwan S. Roles of ribosomal binding, membrane potential and electron transport in bacterial uptake of Streptomycin and Gentamicin. Antimicrobial Agents and Chemotherapy, 1983 ; 23 : 835-845. - CA-SFM. Recommandation 2016. - Cortes G., Lozano-Zarain P., Torres C., Alonso C.A., Ríos-Torres A.M., Castañeda M., López-Pliego L., Navarro A., Del Carmen Rocha-Gracia R.2017 Extended-spectrum β-lactamase-producing Escherichia coli isolated from healthy humans in Mexico, including subclone ST131-B2-O25:H4-H30-Rx. J Glob Antimicrob Resist. 2017 Jun;9:130-134. doi: 10.1016/j.jgar.2017.02.014.	anaerobic bacteria are susceptible to penicillin. c. We doubt the synergistic effect in vivo. d. It is not true that genetic determinants for streptomycin are mainly located on the chromosome. e. This reflection paper is not on penicillin, but on AGs Further subcategorization for AMEG to decide.

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	- Doi Y, Wachino J, Yamane K, Shibata N, Yagi T, Shibayama K, Kato H, Arakawa Y. Spread of novel aminoglycoside resistance gene aac(6')-Iad among Acinetobacter clinical isolates in Japan. Antimicrob Agents Chemother. 2004 Jun;48 (6):2075-80. - Hancock R.E.W. Aminoglycoside uptake and mode of action with special reference to streptomycin and gentamicin.-I Antagonists and mutants. Journal of Antimicrobail Chemotherapy, 1981 ; 8 : 249-276. - Jawets E., Gunnison J.B., Coleman V.R. The combined action of penicillin with streptomycin or chloromycetin on enterococci in vitro. Science ; 1950 ; 111 : 254-256. - Jin W., Wachino J., Kimura K., Yamada K., Arakawa Y. New plasmid-mediated aminoglycoside 6'-N-acetyltransferase, AAC(6')-Ian, and ESBL, TLA-3, from a Serratia marcescens clinical isolate. J Antimicrob Chemother. 2015 May; 70(5):1331-7. doi: 10.1093/jac/dku537. Epub 2015 Jan 8. - Kania B.F., Kania K., Sutiak V. bacterial resistance and clinical effectiveness of combinations of b-lactam with aminoglycoside antibiotic on the example of Vetramycin and Siccovet. Medycyna Wet ; 2005 ; 61 : 746-751. - Kresken M.; Körber-Irrgang B., Läuffer J., Decker-Burgard S., Davies T. In vitro activities of ceftobiprole combined with amikacin or levofloxacin against Pseudomonas aeruginosa: evidence of a synergistic effect using time-kill methodology. Int J Antimicrob Agents. 2011 Jul;38(1):70-5. - Maryam L., A.U. Khan. A Mechanism of Synergistic Effect of Streptomycin and Cefotaxime on CTX-M-15 Type β-lactamase Producing Strain of E. cloacae: A First Report. Frontiers in Microbiology, 2016: 1-11. - Moelling R.C., Wennersten C., Weinberg A.N. Studies on antibiotic synergism against enterococci. I. Bacteriologic studies. Journal of Laboratory and Clinical Medicine ; 1971 ; 77 : 821-828. - Moelling R.C., Weinberg A.N. Studies on antibiotic synergism against enterococci. II. Effect of	

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	various antibiotics on the uptake of "C-labelled streptomycin by enterococci. Journal of Clinical Investigation ; 1971 ; 50 : 2580 -2584. - Mzali F., Butty P., Hernould M., Payet A., Dubois V., Kann. M. Evaluation of Benzylpenicillin/dihydrostreptomycin (Intramicine®, CEVA) against clinically significant veterinary bacterial pathogens. 17th ECCMID (2016) Amsterdam. - Plotz P.H., Davis B.D. Synergism between streptomycin and penicillin: aproposed mechanism. Science; 1962 ; 135 : 1067-1068. - Rosselet A., Schluep J., Knüsel F. A quantitative in vitro evaluation of the combined action of benzylpenicillin and Dihydrostreptomycin on Staphylococci isolated from the bovine udder with special regard to synergistic activities. Zbl. Vet. Med. B ; 1977 ; 24 : 35-52. - Schwarz S., Werckenthin C., Alesik E., Wieler L.H., Wallmann J. Susceptibility of bacterila pathogens against lincomycin/spectinomycin (1/2), penicillin G/neomycin (1/1), and penicillin G/dihydrostreptomycin (1/1) as determined in the Bft-GermVet monitoring program 2004-2006. Berliner und Münchener Tierärztliche Wochenschrift, 2007 ; 120, Heft 9/10 : 363-371. - Springer B., Kidan Y.G., Prammananan T., Ellrott K., Böttger E.C., Sander P. 2001. Mechanisms of Streptomycin Resistance: Selection of Mutations in the 16S rRNA Gene Conferring Resistance. Antimicrob. Agents Chemother. vol. 45 no. 10: 2877-288. Sunde M., Norström M. The genetic background for streptomycin resistance in Escherichia coli influences the distribution of MICs. Journal of Antimicrobial Chemotherapy ; 2005 ; 56 : 87-90. - Vakulenko S.B., Mobashery S. Versatility of aminoglycosides and prospects for their future. Clinical Microbiology Reviews ; 2003 ; 16 : 430-450. - Vet01-S2. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals ; second informational supplement. CLSI ; July 2013. - Watanakunakorn C., Glotzbecker C. Synergism with aminoglycosides of penicillin, ampicillin and vancomycin against non-enterococcal group-D streptococci and viridians Streptococci.	

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	Journal of Medical Microbiology ; 1977 ; 10 : 133-138. - Whittem T., Hanlon D. Dihydrostreptomycin or streptomycin in combination with penicillin G in dairy cattle therapeutics: A review and re-analysis of published data Part 1: Clinical pharmacology. New Zealand Veterinary Journal, Volume 45, Issue 5, pp 178-184, Oct 1997. - Yee Y., Farber B., Mates S. Mechanism of Penicillin-streptomycin synergy for clinical isolates of Viridans streptococci. The Journal of Infectious Diseases ; 1986 ; 154 : 531-534.	
3.	FVE welcomes the reflection paper on aminoglycosides. Veterinarians, represented by FVE, are strongly committed to responsible use of medicine, which has yielded very good results leading to an overall decline of overall antibiotic sales and the sales of CIAs (7th ESVAC report). FVE supports that proper prescription and responsible use of medicines include examination of the animals by a veterinarian, diagnostic testing and diagnosis. Veterinarians are trained to apply the principles of responsible use and aware of the risk of development of resistance to certain products, especially of antibiotics. We, however, have doubts whether AG's as a whole group should be placed in AMEG II or only certain antimicrobials within the AG class. Aminoglycosides in animals are a very wide and varied group, some used a lot, others seldom, used in different species, through different routes of administration and for different aerobe Gram – bacteria. Some are used both in human and animal medicines while others solely in animal or human medicine. Cross resistance exists but there is no complete cross resistance to all AGs. This is also seen by the very varied resistance levels between the different antimicrobials in the AG class. The route of administration in the case of aminoglycosides is very important. When treating an individual animal in a non-systemic way, e.g. topical, the risk is low. While the risk from oral products used to treat enteric infections is much higher. This is also recognised in the reflection paper. We miss two important chapters in the paper, one is a table (similar as table 2) on the	Thank you.

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	importance of AGs in veterinary medicine. Hereby just some examples of where AGs are essential in the veterinary field are e.g. - for post-weaning diarrhoea in pigs and for poultry infections. It needs to be recognised, that the pig sector is seriously affected from the recent measures resulting to restriction in use of colistin and banning of ZnO. - In laying hens (outdoor), neomycin is currently the only alternative to colistin with a withdrawal period of 0 days. Regarding the Campylobacter-hepatitis of laying hens (outdoor) neomycin is the only product which works very efficiently and rapidly. - Aminoglycosides are also considered essential in equine veterinary medicine, in particular given the frequency of infection by gram- bacteria, such as pseudomonas in respiratory problems and other well identified special conditions for which no other alternatives exist. Further to this one should consider that if these products were unavailable it would cause serious health implications in horses. Additionally, the use of AGs in horses and the linked AMR risk are very low. We very much support the paper in saying that veterinary breakpoints need to be set, as due to their current absence, the interpretation of susceptibility testing is impaired. We miss in chapter 6 antibiotic resistance data from humans. AMEG Category 2 includes those antimicrobial classes listed as CIAs by WHO for which the risk to public health from veterinary use is only considered acceptable provided that specific restrictions are placed on their use and that these reserved antimicrobials should be used only when there are no alternative antimicrobials authorized for the respective target species and indication. While we agree that certain antimicrobials within the aminoglycoside class might benefit from putting in the Category 2 class, a further reflection paper is needed on which these antimicrobials should be and what specific restrictions would be beneficial to apply. In the risk	Partly agreed. The importance of AGs in veterinary medicine is already mentioned in the RP. A table does not seem necessary. We added a sentence on the importance of AGs in post-weaning diarrhoea in pigs: "Aminoglycosides are an important alternative to colistin for the treatment of post-weaning diarrhoea caused by *E. coli* in pigs".Campylobacter hepatitis does not seem to be a common condition. Pseudomonas is not a common pathogen in respiratory infections in horses, but the importance of AGs for Gram-negative infections in general in horses is already mentioned in the RP, as is their importance for treatment of pseudomonas infections in general. We added some data on antibiotic resistance of human pathogens. This information is useful for context, however, not essential for the specific assessment of the risk of transmission of resistance from animals to humans. Alternative antibiotics to aminoglycosides are considered in

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	analysis, the risks towards animal health and welfare and those posed by treatment with alternative antibiotics should be taken into account. It is for veterinarians important to being able to access different classes of antibiotics to be able to cure the animals under their care by choosing the optimal antibiotic treatment to a respective bacterial infection. Resistance development by suboptimal treatment has to be avoided. AGs are bactericidal, requiring often high doses for a short term to avoid resistance development. It is important that there is an overall assessment of the efficacy of AGs products with a view to ensure that the available options intended for the treatment of animals are efficacious. We also note that the paper is very critical on the use of combination products, which in some member states are the majority of products authorised and which in practice are used a lot. It would be worth adding that synergy between e.g. penicillin and streptomycin has been demonstrated for various human pathogens, e.g. Streptococci, Enterococci (Watanakunakorn and Glotzbecker 1977, J. Med. Microbiol. 10, 133-138; Torres et al 1995, Eur. J. Clin. Infect. Dis. 14, 878-882). Later on, such synergies have been demonstrated for some veterinary pathogens as well, namely Pasteurella multocida, Mannheimia haemolytica, Streptococcus uberis (Ganière et al, in Journées Nationales GTV-INRA 1999, Nantes, France). This should be acknowledged in the paper. It also must be recognised that in some countries some aminoglycosides are mainly authorised as combination products, e.g. 7 out of the 8 neomycin authorisations in the UK is of a combination product. If combination products would be prohibited, this would have enormous impacts on availability and as a consequence animal health and possibilities for veterinarians to treat their patients.	chapter 8 of the paper. AMEG will decide on criteria for further categorisation. Yes, but this is beyond the scope of the reflection paper. Not agreed. See comments to stakeholder 1. There is no evidence of synergy between penicillin and streptomycin (at current doses and for current indications) *in vivo*. The penicillin/streptomycin combination was withdrawn from the US market in 1993 as no evidence of clinical synergy was presented.
4.	AnimalhealthEurope welcomes the opportunity to comment on this reflection paper. According to the AMEG criteria, veterinary-authorised Aminoglycosides would be placed in Category 2, although the reflection paper acknowledges that Aminoglycosides have a lower risk profile than fluoroquinolones and 3rd and 4th generation cephalosporins. Strict implementation of the AMEG categorisation would mean that in effect all, or nearly all, antibiotics available in veterinary medicine effective to treat Gram-negative infections would be in Category 2. In	AMEG will decide on further categorization.

Stakeholder no.	General comment	Outcome
	relation to the Aminoglycoside group there are possible impacts both on animal health (restricted availability of effective treatments) and on human health (zoonotic infections, risk of Aminoglycosides replacement in animal health by altermatives "that include antimicrobials that are critically important for the treatment of human infections, such as fluoroquinolones and colistin"). In the meantime, the reflection paper considers a further stratification of the categorisation. Classifying Aminoglycosides as different to Category 1 could work, provided that the definition of an intermediate category between current Categories 1 and 2 would ensure a sufficient availability of Aminoglycosides to treat animal infections due to Gram-negative bacteria, consistent with a "One Health" approach. However, further refinement of the categorisation should of necessity integrate the target species as well as the route of administration, to differentiate individual and non-individual treatments and to consider the risk of exposure of animal gut flora. The risk of emergence of resistance in humans from the use of Aminoglycosides by topical, intramammary or injectables routes is considered as low, as animals are treated individually and no impact on the gut flora is expected. Therefore the topical, intramammary and injectable uses of Aminoglycosides should remain in Category 1, whilst oral use of Aminoglycosides could be classified in an intermediate category between current Categories 1 and 2. This is consistent with previous advice from EMA to take into account route of administration in risk assessment (EMA/381884/2014) and with the recent request of the European Commission to the EMA to update its 2014 advice on the impact of the use of antibiotics in animals on public health and animal health. In that request, the points to be addressed include further refinements of the criteria for the categorisation (e.g. including route of administration). In the case of Aminoglycosides, the reflection paper extensively documents the diversity, and in many cases the specificity of resistance mechanisms of the different Aminoglycosides, not to mention the differences in individual Aminoglycosides that are used in human vs. animal medicine. Section 7 of the document is dealing solely with oral use of Aminoglycosides, which further underscores the need for sub-categorisation – not only between fluoroquinolones and 3rd - 4th generation cephalosporins vs Aminoglycosides on the one hand, but also within the	

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	Aminoglycosides based on intestinal exposure, hence route of administration. This reflection paper is slightly disappointing (compared to similar previous reflection papers) in terms of scientific rigour. While this may relate to the fact that there is less published or clear-cut evidence compared to fluoroquinolones and 3rd and 4th generation cephalosporins, this is not clear and this needs to be acknowledged. Notwithstanding this, there are several inconsistencies and inappropriate extrapolations (listed below) which undermine the robustness of the overall arguments and conclusions.	Not agreed.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
26 -27	2.	Comment: Indeed mechanisms conferring resistance to (dihydro)streptomycin and spectinomycin usually differ from those of the other AGs. Please see the § 4, 5 and 6 in the chapter "General comments".	This is clearly stated. No amendment needed.
38-42	2.	Comment: It is not demonstrated that transfer of bacterial resistances initially come from animals or humans. There are contradictions between these 2 lines in some way because it was written that transfer of genetic elements from animals to humans conferring resistance is high towards AGs used in humans (gentamicin, tobramycin and amikacin) but the line 41 precise that such resistance observed in veterinary organisms is scarce. Therefore, it would mean that resistance observed in animals would have small impact on human, as the level of resistance in animals is low. Proposed change: These sentences need clarifying.	The first lines refer to resistance mechanisms to all AGs, including streptomycin, neomycin and kanamycin. The resistance genes and mobile elements found in isolates from animals and humans are the same. The levels of resistance depend on the AG tested and the bacterial species investigated. Therefore there is no contradiction.
45-46	2.	Comment: Treatment of intestinal colibacillosis would not be achieved anymore as the panel of available antibiotics against enterobacteriae is now very restricted since colistin has been ranked as a critical antibiotic. So oral AGs (such as paromomycin) could be one of the sole monotherapy antimicrobial, in the close future to treat such disease in veterinary medicine. Proposed change: Precisions on the usage for example of paromomycin monotherapy for specific diseases should be mentioned.	Partly agreed. We added a sentence on the importance of AGs in veterinary medicine, but did not mention paromomycin specifically.
47	2.	Comment: it is not appropriate to generalize such conclusion to the whole family of AGs. First generation of AG should be separately ranked from the more recent AG generations on the scientific basis as it is clearly in accordance with the reflection paper in the lines 623 to 624 " Resistance to streptomycin and spectinomycin for example is distinct from resistance to gentamycin,	For AMEG to decide on further categorization.

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		kanamycin and/or tobramycin". Proposed change: Oldest generation of Veterinary-authorized AGs would be placed in category 1 for the AG mainly used in Veterinary medicine/ very rarely used in human medicine and for which it is scientifically demonstrated that cross-resistance to newer AGs used in human medicine does not occur (Springer B. et al, 2001). Moreover, the usage of older AG is not risky especially if used through the IV route and for an optimal duration (7 days). Whilst the usage of more critical antibiotics such as third and fourth generation cephalosporins or fluoroquinolones are the main drivers of resistance emergence and spread.	Not agreed
47-55 97-107 1037-1047	4.	Comment: Classifying the Aminoglycosides in Category 2 would mean that nearly all antibiotics available in veterinary medicine effective to treat Gram-negative infections would be in that category, with possible impacts both on animal health (restricted availability of effective treatments) and on human health, the latter being mentioned in lines 1027-1032 (risk of Aminoglycosides replacement by altermatives "that include antimicrobials that are critically important for the treatment of human infections, such as fluoroquinolones and colistin"). As it is acknowledged that Aminoglycosides have a lower risk profile compared to fluoroquinolones and 3rd and 4th generation cephalosporins, the option of a further stratification of the categorization could work, provided that the definition of an intermediate category between current Categories 1 and 2 would ensure a sufficient availability of Aminoglycosides to treat animal infections due to Gram-negative bacteria. Further refinement of the categorisation should necessarily integrate the target species as well as the route of administration, to differentiate individual and non-individual treatments and considering the risk of exposure of animal gut flora (see also comment lines 983-1000). Proposed change: Please add after "further stratification of the categorization": "Such further	For AMEG to decide on further categorisation

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		stratification should preserve the availability of Aminoglycosides for veterinary medicine and particularly consider the route of administration."	
54-55	2.	Comment: We agree, as it is indeed a non-sense to rank all AGs used in veterinary medicine in the same category as highly critical antibiotics such as C3G and C4G. Please see our comment in the chapter "General comments"	For AMEG to decide on further categorisation
89-95	4.	Comment: There is no recognition that Aminoglycosides are considered as CIAs for animals by the OIE. This should be added to complete the picture. Proposed change: Please add on line 95: Aminoglycosides are also classified by OIE as critically important antimicrobials for veterinary medicine.	Agreed. This information was added.
98-99	4.	Comment: "Considering the AMEG criteria, veterinary authorized AGs should be placed in Category 2 given (1) their importance in human medicine and (ii) the high potential for transmission of resistance. " AnimalhealthEurope supports the AMEG categorisation as a science based refinement and adaptation to the WHO CIA list. However, as explained in more detail below in this case we believe the AMEG has attached undue weight to the importance of aminoglycosides in human medicine. The paragraph above this quote (lines 89-90) specifically acknowledges that the WHO does not include Aminoglycosides in the highest priority CIA's. A blanket categorisation of Category 2 for Aminoglycosides is not supported given the distinctions in specificity of the resistance mechanisms. We note that on line 808, the reflection paper acknowledges that the WHO lists spectinomycin (an aminocyclitol) as "important" (rather than "critically important"), because it is not the sole or one of the limited treatment options for serious human disease. This in addition to the differences in use levels in human patients as already stated in the paper. Proposed change: See above, the topical, intramammary and injectable uses of Aminoglycosides should remain in Category 1, whilst oral use of Aminoglycosides could be classified in an intermediate category between current Categories 1 and 2.	For AMEG to decide on further categorisation.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
98	2.	Same comment and proposed change as line 47	Same answer
106-107	2.	Same comment as lines 54-55	Same answer
112-116	2.	Comment: Old combination such as (dihydro)streptomycin /benzylpenicilline still efficient (M'Zali F. et al, 2016) against streptomycin resistant bacteria. Such combination is not used in humans; do not overlap with resistance observed with recent AGs. Please see our comment in the chapter "General comments" § 4 ; 5 and 6. Proposed change: Remove the sentence as combinations therapies including AG and old beta-lactams antibiotics (narrow spectrum) combinations is a mainstay widespread empirical therapy (Brilene T. et al, 2013; Kresken M. et al, 2011; Maryam L. et al, 2016).	Not agreed. See our response to the general remarks.
112-116	3.	Comment: Synergy of combination products has been demonstrated and should be acknowledged in the paper. Proposed change (if any): The rationale for the indications for some VMPs containing fixed combinations of AGs, or combinations with antimicrobials from other classes, is questionable. Although synergy between e.g. penicillin and streptomycin has been demonstrated for various human pathogens, e.g. Streptococci, Enterococci (Watanakunakorn and Glotzbecker 1977, J. Med. Microbiol. 10, 133-138; Torres et al 1995, Eur. J. Clin. Infect. Dis. 14, 878-882) as well as some veterinary pathogens, namely Pasteurella multocida, Mannheimia haemolytica, Streptococcus uberis (Ganière et al, in Journées Nationales GTV-INRA 1999, Nantes, France), nowadays there are references of widespread resistance. 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.	Not agreed. See response to general comments above.
121-123	4.	Comment: It is recommended that use of (dihydro)streptomycin and spectinomycin should be based on susceptibility testing while it is	Not agreed. Although we agree that the interpretation of susceptibility

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		acknowledged elsewhere that available tests for Aminoglycosides are "problematic for many bacterial species" (line 619). Indeed, a need for research is mentioned to enable the proper interpretation of susceptibility tests (lines 130-131: standardisation of tests and definition of veterinary clinical breakpoints). Therefore, such research should precede possible implementation of susceptibility tests to base the use of these compounds. Proposed change: Please add after Lines 121-123, "However such recommendation is dependent on prior validation of reliable susceptibility tests for AGs (see below Needs for research)".	testing could be improved, this does not mean that recommendations or implementation should be postponed.
136	4.	Comment: VetCAST is listed as a responsible party while this institute has, to date, no standards, guidelines, SOPs etc. VetCAST may in the future achieve its stated aims but currently has hardly reached a pilot stage and, as yet, there is no realistic target date to reach this level of maturity. This reflection paper should focus on statutory bodies or third-party organisations with a proven track record of delivery. Proposed change: It is not appropriate to reference VetCAST at this time. Please delete reference to VetCAST. Reference should be made to other sources which do exist such as CLSI VAST.	Not agreed. VetCAST is a EUCAST subcommittee.
255 – 292	4.	Comment: In this section, the reflection paper appears to rely on submissions from one or a very limited selection of NCAs to describe use across Europe. This approach has certain limitations and may not be representative for the situation across Europe. Preferably, a complete assessment should be made, or a targeted sample covering the majority of the animal population in a particular species. In our view, extrapolating the status in Norway to the overall European pig herd is suboptimal. Proposed change: We suggest reworking this section to provide a more representative picture of the EU as a whole.	Agreed. We added information where necessary. A complete assessment is unnecessary.
316-317	2.	Comment: We disagree, as it is not appropriate to write that no rational exist for the oldest combinations including benzylpenicillin/ (dihydro)streptomycin. Mechanisms of actions and impact on antibiotic resistance had been	Not agreed. The rationale is given in lines 293-4, later text advises that the rationale is disputable. See

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		demonstrated in the scientific literature: "The synergistic activity of this combination has even been observed on microorganisms which have developed resistance to both of these molecules (Rosselet A. et al, 1977; Kania B.F. et al, 2005). The mode of action of the synergistic effect of this combination is based on the penicillin-induced lysis of the micro-organism's membrane which makes it easier for streptomycin (C14-labelled in the study) to enter the cell and promotes an action on the intracellular ribosomal sites which is more direct and more effective compared with the use of streptomycin alone (Plotz P.H. et al, 1962). This activity has also been observed both on gram-negative bacteria such as E. coli (Plotz P.H. et al, 1962) and on Gram + micro-organisms (involving an additional effect on the walls of these microorganisms (Moelling R.C. et al, 1971; Watanakunakorn C. et al, 1977). The prevalence of MDR bacteria remains low therefore, in order not to compromise the usage of wider spectrum antimicrobials, benzylpenicillin/DHS could be used as first line therapy to overcome resistant bacteria to either streptomycin or penicillin as recommended by a recent published study (M'Zali F. et al, 2016). Proposed change: Modified the sentence "The rational for some of these combinations is disputable as some include molecules have not proven to be synergistic, which is not the case for beta-lactam/old AG combinations such as benzylpenicillin/DHS) as the association of a narrow spectrum beta-lactam and an aminoglycoside (Whittem T. et al,.1997).	comments above.
318	2.	Comment: It is not appropriate to write that generally all combinations have a very limited extra value as some of them are widely used in veterinary medicine (volume of use of dihydrostreptomycin = 129 tonnes in 2014 : table 1) in the time when many antibiotics are classified as critical. Furthermore, efficacy of benzylpenicilline/DHS against bacteria resistant to dihydrostreptomycin had been again recently re-demonstrated (M'Zali F. et al, 2016). Proposed change: We propose to remove the terms "streptomycin-penicillin combination" in the sentence.	The fact that the combination is widely used is no argument to ensure that this is responsible use. Significant reductions were possible in certain MS without affecting animal health and welfare. Not Agreed. There is no evidence of synergy between penicillin and streptomycin (at current doses and for

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
			current indications) *in vivo*. There is no clinical information that supports that the penicillin-streptomycin combination offers any advantage compared to penicillin alone for the main indications. The penicillin/streptomycin combination was withdrawn from the US market in 1993 as no evidence of clinical synergy was presented.
318-319	4.	Comment: Following demonstration of synergy between penicillin and streptomycin on various human pathogens: Streptococci, Enterococci, Lactobacilli (Watanakunakorn and Glotzbecker 1977, J. Med. Microbiol. 10, 133-138; Bayer et al 1980, Antimicrobial Agents Chemother. 17, 359-363; Yee et al 1986, J. Infectious Dis. 154, 531-533; Torres et al 1995, Eur. J. Clin. Infect. Dis. 14, 878-882), such synergy has also been shown on various veterinary pathogens: Pasteurella multocida, Mannheimia haemolytica, Streptococcus uberis (Ganière et al, in Journées Nationales GTV-INRA 1999, Nantes, France). Proposed changed: Modify the last sentence by "However, a synergistic effect of this combination has been shown for a range of different pathogens and bacterial strains."	Not agreed. See above.
322	1.	Comment: The use of streptomycin against fire blight caused by Erwinia amylovora in orchards should be mentioned here. Proposed change (if any): Streptomycin (and in rare cases also gentamicin) is also widely used to control fire blight caused by *Erwinia amylovora* in orchards (Stockwell VO, Duffy B.	Agreed. Sentence was added.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Use of antibiotics in plant agriculture.Rev Sci Tech. 2012 Apr;31(1):199-210. Review.PMID:22849276).	
327	2.	Proposed change: Please precise if the tonnage linked with DHS in the table 1 also concerned the use of DHS in the combination of benzylpenicillin/DHS	Agreed. A footnote was added to clarify this.
481	3.	Comment: A table is missing on the importance of AGs in veterinary medicine. Proposed change: include a table on importance of AGs in veterinary medicine.	Table not needed. Importance discussed in the text.
501	1.	Comment: *baumanii* is not correctly written Proposed change (if any): Please replace *baumanii* with *baumannii*	Agreed. Spelling was corrected.
530	1.	Comment: AAC(6')-Ie-APH(2")-Ia has also been found recently in other Gram-positive bacteria like Macrococcus (Cotting K, Strauss C, Rodriguez-Campos S, Rostaher A, Fischer NM, Roosje PJ, Favrot C, Perreten V. Macrococcus canis and M. caseolyticus in dogs: occurrence, genetic diversity and antibiotic resistance. Vet Dermatol. 2017 Jul 26. doi: 10.1111/vde.12474. [Epub ahead of print], PMID: 28748533). It is very likely that it has also been found inother Gram-positive bacteria and the list should not be exhaustive. Proposed change (if any): The sentence should be modified as follows: "This enzyme has been found in Gram-positive bacteria like e.g. *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Macrococcus* spp., and *Lactobacillus* spp.."	Agreed. Macrococcus spp. was added.
545	1.	Comment: ant(6) should be ant(6)-Ia Proposed change (if any): Change the sentence to: "The *ant(6)* gene is often found in a cluster *ant(6)-Ia-sat4-aph(3')-III* that specifies resistance to AGs and streptothricin.	Agreed. Change made.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
568	1.	Comment: rmtH has been identified in Klebsiella in 2012. Proposed change (if any): Please add *rmtH* in the list after *rmtG* and add reference: O'Hara JA, McGann P, Snesrud EC, Clifford RJ, Waterman PE, Lesho EP, Doi Y.Novel 16S rRNA methyltransferase RmtH produced by *Klebsiella pneumoniae* associated with war-related trauma.Antimicrob Agents Chemother. 2013 May;57(5):2413-6. doi: 10.1128/AAC.00266-13. Epub 2013 Mar 11.PMID: 23478957	Agreed. *rmtH* has been added.
573	1.	Comment: Include a more general reference Proposed change (if any): Include the following reference: Berçot B, Poirel L, Nordmann P. Updated multiplex polymerase chain reaction for detection of 16S rRNA methylases: high prevalence among NDM-1 producers. Diagn Microbiol Infect Dis. 2011 Dec;71(4):442-5. doi: 10.1016/j.diagmicrobio.2011.08.016. Epub 2011 Oct 13.	Agreed. Reference added.
577-580	1.	Comment: Use a standard nomenclature for consistency. Proposed change (if any): L577: Replace *aacA/aphD* with *aac(6')-Ie-aph(2")-Ia* *L578: Replace aadD with ant(4')-Ia* L579: Replace *aphA3* with *aph(3')-III*	Agreed.
589	1.	Comment: The armA gene has been found in *A. baumannii* strains which also produce a carbapenemase. Proposed change (if any): We propose to modify the sentence as follows and include references: "In *Acinetobacter baumannii*, the *armA* gene, located on a transposon, is widespread in many countries worldwide, and has also been found to be associated with a carbapenemase gene like bla_{OXA-23} and bla_{NDM-1} (Potron et al.,	Not agreed. This chapter is on resistance mechanisms, not on the occurrence of resistance. RP is mainly on situation in EU MS. Reference is on Acinetobacter in

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		2015).” Add the following reference: El-Sayed-Ahmed MA, Amin MA, Tawakol WM, Loucif L, Bakour S, Rolain JM. High prevalence of bla(NDM-1) carbapenemase-encoding gene and 16S rRNA *armA* methyltransferase gene among *Acinetobacter baumannii* clinical Isolates in Egypt. Antimicrob Agents Chemother. 2015;59(6):3602-5. doi: 10.1128/AAC.04412-14. Epub 2015 Mar 23.PMID:2580156.	Egypt.
607 Table 3	1.	Comment: Please add the occurrence for ANT (4’) and ArmA	The occurrence was deleted for all genes, as the situation might vary per country ans systemic surveillance of these resistance genes is not routinely performed.
607 Table 3	1.	Comment: Please capitalize the Methyltransferases since they are proteins. Proposed change (if any): ArmA RmtA, RmtB, RmtC, RmtD, RtmD2, RmtE, RmtF, RmtG NpmA	Agreed.
645	3.	Comment: Chapter 6 is missing occurrence in resistance in bacteria from humans Proposed change (if any): Include extra chapter on the resistance in humans.	Agreed. A chapter on the occurrence of AG resistance in humans is added, although this information provides context, it is not needed for the assessment of the risk of transfer of resistance from animals to humans.
646-744		Comment: The elaboration of percentages of resistance based on various countries and surveys is difficult to read, and doesn’t provide a systematic review of resistance among animals. The review doesn’t consider sampling structure, or numbers of isolates, source of isolates, breakpoints and, where	The percentages of resistance included are mainly those of countries with surveillance systems in place and data of the EFSA

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		appropriate the resistance mechanism. Given the specificity of resistance mechanism within the Aminoglycoside group, this level of detail becomes important to understand the data available. Proposed change: Please provide the data in tabular format, noting with critical evaluation whether any particular survey cited is representative of the region specified.	surveillance. We do not think that a table is helpful.
692	1.	Comment: The Perez reference to support the position that "It should be noted that even bacteria causing human infections not directly linked to animals may acquire resistance determinants from bacteria with zoonotic potential" is inappropriate. While there was molecular evidence of homologous sequences between P. aeruginosa from humans isolates and Salmonella species generally, it is not sufficient evidence of genetic recombination between animal-based sources, to suggest that "the indirect risk from the use of AGs in food animals should therefore be taken into account in determining risk profiles. The Perez study examined 7 isolates from (quoting the study authors) "patients who were affected (that) had multiple comorbidities, endured prolonged colonization, required long-term care, and, in one instance, had a lethal outcome from a bloodstream infection." The epidemiological link among these genetically related isolates appears to be admission to a community hospital and residence in long-term-care facilities in northeast Ohio, except for a patient who was transferred to the tertiary medical center from Qatar. To extrapolate this study in Ohio, to risk of AG use in food animals in the EU is not appropriate for purposes of risk categorisation or mitigation. Proposed change: Please rephrase this paragraph.	The sentence was moved in order to make clear that the Perez reference is not the only reference to support this statement.
719	1.	Comment: While unsurprisingly there is an occupational hazard for people working closely in contact with animals of clonal transmission in either direction, this should not be extrapolated to the population as a whole. This paragraph relies on experience on farms but then generalises to the population as a whole. Therefore this section is a risk for farm staff but only really a hazard for the	If transmission between animals and humans is possible at a farm, the risk of transmission, although not quantified, is also present in the general population. Quantitative

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		wider human population. Any farm data should be put in context with clarity on whether this has been shown to be a risk for the wider human population or is still unquantified/unproven. Studies of the epidemiology of certain drug classes should not be used as evidence of other drug classes. If indeed this were the case, then one would expect more even distribution of resistance profiles among the drug classes, when this clearly not the case.	data on transmission are lacking.
720	1.	Comment: 5405 from transposon Tn5405 should be in italic Proposed change (if any): L720: Replace Tn5405 with Tn5405	Agreed.
735	1.	Comment: Please add the genes for consistency with the next sentence on L738. Proposed change (if any): L733-736: Replace the actual sentence with the following one including the genes: "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).	Agreed.
738	2.	Comment: The genes mentioned in these lines do not concern dihydrostreptomycin. Proposed change: Addition of "(AGs) except for dihydrostreptomycin.	Not agreed. Although armA does not confer resistance to streptomycin, most isolates in the study were streptomycin resistant
771-774	2.	Comment: Dihydrostreptomycin does not play an active role in the spread of genetic determinants encoding ESBLs as the resistance determinants involved in the ESBLs spread are those of third/fourth generation cephalosprins, quinolones and the newer generations AGs all of which are heavily used in human medicine. Proposed change: Exception regarding dihydrostreptomycin should be added.	Not agreed. AGs can select for ESBL resistance determinants through co-selection and streptomycin is no exception.
795-798	2.	Comment: AGs such as paromomycin are important drugs for oral application	Not agreed. There are alternative

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		against susceptible strains E.coli causing post-weaning diarrhoea in calves and pigs (Whittem T. et al, 1997). Suggested alternatives are very poorly efficient against such pathogens, aminopenicillins are under evaluation by EMA and there is actually an attempt by the WHO to ban C3G, C4G cephalosporins, quinolones and colistin in veterinary medicine. Some AGs could be the one of the only way to treat livestock animals against such diseases and to avoid a critical sanitary crisis for human consumers.	treatments for most infections in animals, although it is acknowledged that prevalence of resistance to many of these substances is high in some infections caused by Gram negative bacteria and in these cases the alternative may be a substance in AMEG's category 2 (chapter 8).
806	2.	Comment: We disagree, because it is a not scientifically rational to categorize streptomycin at the same level as kanamycin and the newer generations AG. Proposed change: please remove streptomycin from the list	Criteria for the categorisation of antimicrobials and the need for further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG).
825	1.	Comment: Worldwide dissemination of aminoglycoside and carbapenem-resistant *A. baumannii* is a major threat. Proposed change (if any): Include *Acinetobacter* into the sentence and modify as follows: In recent years, the global dissemination of *A. baumannii* and Enterobacteriaceae, including *Salmonella* spp., that co-produce 16S-rRNA methylases and carbapenemases is becoming a serious threat to human health. In Enterobacteriaceae, the resistance genes are often co-located on the same plasmid.	Agreed.
832-834	2.	Proposed change: Such affirmation is important. Illustration and references are required.	The comment is acknowledged and the sentence has been deleted.
880-886	4.	Comment: The Perez reference to support the position that "It should be noted that even bacteria causing human infections not directly linked to animals may acquire resistance determinants from bacteria with zoonotic potential" is inappropriate. While there was molecular evidence of homologous sequences between P. aeruginosa from humans isolates and Salmonella species generally,	See response to comment above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		it is not sufficient evidence of genetic recombination between animal-based sources, to suggest that "the indirect risk from the use of AGs in food animals should therefore be taken into account in determining risk profiles. The Perez study examined 7 isolates from (quoting the study authors) "patients who were affected (that) had multiple comorbidities, endured prolonged colonization, required long-term care, and, in one instance, had a lethal outcome from a bloodstream infection." The epidemiological link among these genetically related isolates appears to be admission to a community hospital and residence in long-term-care facilities in northeast Ohio, except for a patient who was transferred to the tertiary medical center from Qatar. To extrapolate this study in Ohio, to risk of AG use in food animals in the EU is not appropriate for purposes of risk categorisation or mitigation. Proposed change: Please rephrase this paragraph.	
887-895	4.	Comment: While unsurprisingly there is an occupational hazard for people working closely in contact with animals of clonal transmission in either direction, this should not be extrapolated to the population as a whole. This paragraph relies on experience on farms but then generalises to the population as a whole. Therefore this section is a risk for farm staff but only really a hazard for the wider human population. Any farm data should be put in context with clarity on whether this has been shown to be a risk for the wider human population or is still unquantified/unproven. Studies of the epidemiology of certain drug classes should not be used as evidence of other drug classes. If indeed this were the case, then one would expect more even distribution of resistance profiles among the drug classes, when this clearly not the case.	See response to stakeholder 1.
896-900	4.	Comment: The genetic linkage of resistance determinants from Enterobacteriaceae isolates from pets in China is not sufficient evidence in and of itself to extrapolate risk of transmission broadly between animals and humans, especially when the quoted publication (Deng et al., 2011) acknowledges that transfer between animals and humans was not studied.	Not agreed. The paragraph is on transmission of resistance determinants between animals and humans. It is not on occurrence or prevalence of resistance. If

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: Please delete the paragraph.	resistance determinants have been found on plasmids they can potentially be transferred. This is not only true for China. Therefore the paragraph was kept as it is.
929	2.	Comment: According to table 2, streptomycin as well as (dihydro)streptomycin are very rarely used in human compared to the other AGs. It would be appropriate to note this point. Proposed change: add "except for apramycin which are only used in animals and (Dihydro)streptomycin which is very rarely used in humans."(Table 2).	Streptomycin is rarely used in humans in EU MS. This is already mentioned in the RP. It is sometimes used in multidrug resistant tuberculosis. Apramycin can cause cross-resistance to gentamicin.
964-965	2.	Comment: It has been written several times in the reflection paper that the oldest generation of AGs almost exclusively used in animals represented by (Dihydro)streptomycin and spectinomycin presents differences to the other AGs. This sentence confirms once more the requirement to separate the ranking of such molecules from the remaining AG generations .	Criteria for the categorisation of antimicrobials and the need for further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG).
983-1000	4.	Comment: The assessment of the risk for the emergence of resistance in humans from the use of Aminoglycosides in animals is detailed per route of administration. The topical routes are considered at low risk as they concern individual treatments and they do not impact the gut flora. It can be added that high concentrations of Aminoglycosides are reached locally by use of these topical routes insuring a full bactericidal action for concentration dependent antibiotics such as Aminoglycosides. The same assessment is made for the intramammary route. For this route of administration, it can be emphasized that the udder, generally sterile and even infected, is not a site favourable to genetic exchanges between bacteria,	Criteria for the categorisation of antimicrobials and the need for further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG).

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		contrary to the digestive tract where the bacterial burden and diversity are far higher. Moreover, no resorption of Aminoglycosides is expected from the udder, meaning no impact on the gut flora. Regarding the injectable route, the risk is considered lower if animals are treated individually. It can be added that as Aminoglycosides are eliminated via the renal route (Prescott J.F. et al, Antimicrobial Therapy in Veterinary Medicine, 2000, Third Edition, Iowa State University Press), no impact of treatment by injection is expected on the gut flora. Therefore, the route of administration appears an important criterion for the risk assessment and subsequent categorization of Aminoglycosides. This is consistent with previous advice from EMA to take into account route of administration in risk assessment (EMA/381884/2014) and with the recent request of the European Commission to the EMA to update its 2014 advice on the impact of the use of antibiotics in animals on public health and animal health. In that request, the points to be addressed include further refinements of the criteria for the categorisation (e.g. including route of administration).	
1001-1002	3.	Comment: Aminoglycosides are also considered essential in equine veterinary medicine, in particular given the frequency of infection by pseudomonas. Proposed change: In veterinary medicine, AGs are one of the few treatment options for considered essential in equine veterinary medicine, in particular given the frequency of infection by gram - bacteria and especially Pseudomonas infection for which no alternative treatment is availableand for infections with Gram-negative bacteria in horses.	This is already mentioned in the RP and Pseudomonas infections are uncommon in horses.
1006	4.	Comment: The statement that risk of transmission of resistant Enterobacteriaceae to humans from non-human sources is regarded as high is not referenced and certainly this was the view in 2011, but since then whole genome sequencing has suggested that in fact this may not be the case (de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, et al. (2014) PLoS Genet 10(12): e1004776. doi:10.1371/journal.pgen.1004776) Proposed change: Please update this section considering more recent	This reference only refers to a small number of ESBL-producing E. coli isolates, not to other Enterobacteriaceae such as Salmonella spp.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		publications.	
1027-1028	3.	Comment: Proposed change: If AGs were no longer available for veterinary medicine then it could be expected speculated that other critical antimicrobials would replace their use.	Criteria for the categorisation of antimicrobials and the need for further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG).
1037-1047	2.	Comment: A sub-classification of AG molecules should be set up as it is indeed a nonsense to rank all AGs in the same category as highly critical antibiotics such as C3G and C4G Knowing that the oldest AGs such as (dihydro)streptomycin exhibit different and specific resistance mechanisms not inducing collateral damages to the other AG. Streptomycin should be kept as first line therapy away from the more potent AGs (in accordance with lines 623-624 and 576 of the reflection paper). Proposed change: Maintain in Category 1 old AGs such as (dihydro) streptomycin rarely used in human medicine compared to spectinomycin (table 2, lines 415, 430-431, 576, 623-624 of the reflection paper) and Benzypenicillin/(dihydro)streptomycin combination, especially for parenteral applications. In the light of this data, we suggest that benzylpenicillin/DHS combination could be a useful first line empirical therapy in veterinary medicine. As it is indeed a combination of two narrow spectrum antibiotics, its enhanced activity in comparison to penicillin G or streptomycin alone, against a wide range of bacterial pathogens, positions this drug as a good candidate for first line therapy in veterinary medicine. More importantly, benzylpenicillin/DHS usage will not compromise a subsequent use of extended spectrum aminoglycosides or beta-lactam antibiotics both in human and veterinary settings.	Criteria for the categorisation of antimicrobials and the need for further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG). Not agreed. See response above.
1044-1047	3.	Comment: The conclusions should be adapted and include an overall impact assessment to both human and veterinary medicine prior to recommendation	Criteria for the categorisation of antimicrobials and the need for

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		and implementation of a reclassification of AGs products, while recognising that both doctors and veterinarians are highly trained professionals. Proposed change: …it is recommended that an overall impact assessment for veterinary-authorised AGs should be conducted. A further reflection paper on which of these antimicrobial products should be in such a list and what specific restrictions would be beneficial to apply should be developed. In the risk analysis, the risks posed by treatment with alternative antibiotics should also be taken into account. could be placed in Category 2, although the AMEG could give consideration to a further stratification of the categorization.	further stratification will be discussed by the Antimicrobial Advice *ad hoc* Expert Group (AMEG).
1050	3.	Comment: Proposed change: 2 additional references to be added: 1. Watanakunakorn and Glotzbecker 1977, J. Med. Microbiol. 10, 133-138; Torres et al 1995, Eur. J. Clin. Infect. Dis. 14, 878-882 2. Ganière et al, in Journées Nationales GTV-INRA 1999, Nantes, France	Not agreed. Very old references, no added value.