17 February 2021
EMA/CVMP/AWP/902538/2019
Committee for Medicinal Products for Veterinary Use

Overview of comments received on 'Reflection paper on the use of aminopenicillins and their beta-lactamase inhibitor combinations in animals in the European Union: development of resistance and impact on human and animal health' (EMA/CVMP/AWP/842786/2015)

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

Stakeholder no.	Name of organisation or individual
1	The EUCAST Veterinary Subcommittee on Antimicrobial Susceptibility Testing (VetCAST)
2	AnimalhealthEurope
3	Italian Ministry of Health – Directorate General for Animal Health and Veterinary Medicinal Products – Italian CVO
4	Danish Medicines Agency

Comments submitted in reference to this reflection paper but related to the AMEG's advice on 'Categorisation of antimicrobials' (marked with grey) were considered during the development of AMEG's advice and answered in the 'Overview of comments' document specific to the AMEG advice that was published in December 2019 (EMA/CVMP/CHMP/238375/2019).

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1. General comments – overview

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2	AnimalhealthEurope welcomes this well-written and balanced Reflection Paper and appreciates the opportunity to provide comments. This reflection paper relies heavily on data on penicillin and on occasion there is confusion between the older generation penicillins that should not be part of the scope of this reflection paper as well as cephalosporins (*cf* to the title of the present Reflection paper) when the discussion is about aminopenicillins.	Thank you. Not agreed. The focus of the RP is on aminopenicillins, however in certain sections e.g. resistance mechanisms, other penicillins are included because there is cross-resistance between different penicillins.
3	As the Directorate General for Animal Health and Veterinary Medicinal Products of the Italian Ministry of Health, we are pleased to comment on the draft "Reflection paper on the use of aminopenicillins and their beta-lactamase inhibitor combinations in animals in the European Union: development of resistance and impact on human and animal health" https://www.ema.europa.eu/documents/scientific-guideline/reflection-paper-use-aminopenicillins-their-beta-lactamase-inhibitor-combinations-animals-european_en.pdf The comments have been drafted in collaboration with experts of the National Reference Laboratory for Antimicrobial Resistance (Reg. 2004/882/EC) and with the Italian members of the EMA CVMP. The paper has several merits by providing an update on usage of aminopenicillins in Veterinary Medicine for target veterinary bacterial pathogens, mechanisms of resistance, as well as risks for human health arising from usage of such antibiotics in animals. However, in the current version, we find that the Conclusions of the paper are unfounded in scientific evidence, and the paper, as far as	Partly agreed. The section on AMEG has been deleted and all comment have been addressed in the comments to the AMEG advice https://www.ema.europa.eu/en/documents/comments/overview-comments-categorisation-antibiotics-european-union-answer-request-european-commission_en.pdf Comments on macrolides will not be addressed as these are not part of the aminopenicillin reflection paper. Comments on JIACRA are also not within the scope of this RP. In the manuscript the section on AMEG had been replaced by: "Aminopenicillins without beta-lactamase inhibitors are in AMEG category D. It is acknowledged that aminopenicillins are not devoid of negative impact on resistance development and spread, in particular through co-selection. Therefore, while there are

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	"recommendations" and "risk management options" are concerned, remains unclear. In the last sentence of the reflection paper, it reads: "All these factors should be taken into account for the AMEG's categorisation, which is currently under review. It is suggested that the AMEG could give consideration to a further stratification of the categorisation to allow a distinction in the ranking between those substances currently in Category 2 (fluoroquinolones, 3rd- and 4th-generation cephalosporins and colistin, for which there are fewer alternatives) and the amoxicillin-clavulanate combinations, and between the latter and the straight aminopenicillins. Amoxicillin-clavulanate has a wider spectrum and thus it is likely that it has higher chance to select multidrug resistant organisms compared to aminopenicillin alone." At the same time, we have learned that the new AMEG categorisation is under discussion, where the draft report has been submitted for consideration to EMA CVMP/CHMP: it includes four categories: A: "Avoid"; B: "Restrict"; C: "Caution"; and D: "Prudence". It is evident that the Reflection Paper and the new AMEG categorisation are now inextricably linked. For this reason, we have chosen to comment on the Reflection paper in light of the provisional AMEG perspective, commenting on what we have learned on the draft new AMEG classification. In general, we cannot support the option of classifying aminopenicillins in the same (lowest) "risk category for human health" as narrow-spectrum penicillins (i. e. penicillin G, penicillin V, penethamate) as it appears from the draft new AMEG classification (they have been both proposed in the "lowest risk category", Category D). No scientific evidence is provided in either papers to support the use of aminopenicillins in the lowest risk category.	no specific recommendations to avoid use of Category D substances, there is a general recommendation that responsible use principles should be adhered to in everyday practice to keep the risk from use of these classes as low as possible. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment should be restricted to situations where individual treatment is not feasible. Narrow spectrum penicillins with a lower risk of AMR selection should be used for first line treatment where susceptibility testing suggests the likely efficacy of this approach. Aminopenicillins with beta-lactamase inhibitors (e.g.amoxicillin-clavulanate) have a wider spectrum of action and thereby a higher selection pressure for multidrug resistant organisms. Aminopenicillins with enzyme inhibitor are therefore in category C ("Caution") rather than in category D. *The consumption of amox-clav should remain low particularly in food-producing animals and used with caution in all animal species. These substances should only be used when there is no available substance from category D that would be clinically effective. Based on high levels of resistance in Enterobacterales it is recommended that the use of aminopenicillins for the treatment of infections caused by such pathogens should be based on susceptibility testing.* The last paragraph of the summary was slightly amended and now reads:

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	Instead, we propose that further stratification of beta-lactam antibiotics (including recommendations, and how their use should be managed) is advisable within the AMEG classification. For instance, a five-category stratification (and recommendations for each category) may be discussed at the AMEG level in order to provide a better ranking of different beta-lactam antibiotics (i. e. narrow-spectrum penicillins, extended-spectrum penicillins, potentiated aminopenicillins, first and second-generation cephalosporins, third-fourth generation cephalosporins). It is worth pointing out that the EU epidemiological evidence points to a spread of extended-spectrum beta-lactam resistance in Enterobacteriaceae in animals, especially resistance to 3rd & 4th generation cephalosporins (herd-prevalence of ESBL/AmpC-producing *E. coli* in the intestines of food-producing animals is around 50-90% in many EU countries). See "The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food" (EUSR AMR 2015: fattening pigs and veal calves, EUSR AMR 2016 broilers and turkeys). https://www.efsa.europa.eu/en/efsajournal/pub/4694 (EUSR AMR 2015) https://www.efsa.europa.eu/en/efsajournal/pub/5182 (EUSR AMR 2016) Briefly, a suitable option is to classify aminopenicillins in a category of "intermediate risk", i.e. one-step lower that potentiated aminopenicillins (in Veterinary medicine= amoxicillin+ clavulanic acid). Here we further observe that the term "lower" does not mean "low", since the amount of usage of aminopenicillins (and the pattern of usage) in food-producing animals in the EU is high, while it is very high in certain MSs, and the selection pressure exerted by aminopenicillins is highly relevant. In some EU countries (including Italy), sales (in mg/PCU) of aminopenicillins can be 200 times higher than those of 3rd & 4th generation cephalosporins.	"Although the direct AMR risk to humans from the veterinary use of aminopenicillins would be *estimated to be lower* compared to the risk from their use in human medicine, it is evident that veterinary aminopenicillin use increases the selection pressure towards AMR and jeopardizes at least animal health and welfare. *Management strategies to prevent or control related infections and to minimize impacts on animal health and welfare* include improvements in hygiene in animal husbandry, use of vaccinations, proper diagnostics and avoidance of prophylactic use of antimicrobials in animals. Also, the route of administration should be considered to reduce the selection pressure on the gut microbiota. For example, group medication of food-producing animal herds/flocks by the oral route facilitates the selection and spread of resistance and attempts should be made to reduce such use. *In addition, narrow-spectrum penicillins with a lower risk of selection for resistant bacteria should be used for first line treatment wherever possible. Based on an assessment of current use and resistance profiling, it is recommended to limit the further development of resistance to both aminopenicillins and related subclasses of antimicrobials and to maintain the efficacy of these valuable drugs in the future. Prudent use of aminopenicillins as well as other antimicrobials is essential in order to achieve this.* We deleted the term "first line" treatment of animals with aminopenicillins in order to avoid the impression that aminopenicillins are first line treatments.

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	This implies that higher levels of awareness should be raised to animal primary productions, and among veterinary practitioners. Actions should to be taken to reduce the use of aminopenicillins, which are "typically" used by oral route and for "mass medication" (including prophylaxis/metaphylaxis), in food-producing animals. In general, our opinion is that the reflection paper should be more balanced, including points of raising awareness to the possible misuse of aminopenicillins in an animal Health and a One Health perspective. In some critical points, the reflection paper also relies on what can be perceived as opinions of the colleagues whom drafted the manuscript, instead of all statements supported by evidence-based medicine. For example, the reflection paper strongly suggests that aminopenicillins are "first line" beta-lactams. In the Summary (line 72), and in the Discussion (line 1472) it reads that aminopenicillins "are used as first-line" for a number of veterinary pathogens... However, this does not mean that aminopenicillin usage as first-line antimicrobials in Veterinary Medicine is appropriate under any circumstances. It is worth pointing out fundamental differences in the use of aminopenicillins in veterinary versus human medicine. For example, in food animals a sizeable proportion of aminopenicillins are given either in tonnes of bulk animal feed or the common drinking water supply (premixes, oral powders/granules/solutions for drinking water), whereby healthy food animals are NOT separated from diseased ones. Reasons and rationale for not using aminopenicillins as a first-line antibiotic in Veterinary Medicine should have been addressed and sufficiently explained in the reflection paper. Additionally, statements about aminopenicillin usage often mix veterinary bacterial pathogens with human bacterial pathogens, which is somewhat	We separated the sections on humans and animals more clearly to avoid confusion. We did not include a section on susceptibility testing of staphylococci, because this was out of the scope of this reflection paper and we would have to include this information for other microorganisms as well.

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	confusing for the reader. The reader is expecting to find arguments and recommendations for rational and prudent use "in animals", although with a "One Heath" perspective. In the EU food-producing animals (especially in poultry and pigs) aminopenicillins are typically administered via the oral route and for mass medication (or group administration, which in the EU *real world* means they are often administered for metaphylaxis, and even for prophylaxis). For instance, in Italy, sales of the entire group of "penicillins", represent 25% of total sales in the food-producing animals sector (see also Figure 1, and Figure 2 of the Reflection Paper. In Figure 2, it is evident that within the EU some 88% of "penicillins" sold are indeed "aminopenicillins"). This is a valid reason why we also suggest separating aminopenicillins from natural penicillins based on the sales distributions reported in the ESVAC and JIACRA Reports. *In our opinion the reflection paper should highlight that "Aminopenicillins should be considered as first line beta-lactams only for diseases caused by some Enterobacteriaceae: Salmonella (when it is clinically appropriate or when it is allowed by the EU legislation: see Salmonella and poultry, where the use of antimicrobials for the control of Salmonella in poultry is forbidden), Proteus mirabilis, and Yersinia pseudotuberculosis. The latter two bacterial species do not significantly impact animal health and animal primary productions (and most of other Enterobacteria are inherently resistant). Previously, aminopenicillins could be considered as first-line beta-lactams also for E. coli, but since at present there is so much variation in antimicrobial susceptibility of E. coli involved in infectious diseases in food animals (e. g. colibacillosis), then culture and antimicrobial susceptibility results are necessary for prudent antimicrobial choices for diseases caused by E. coli. As for other major Gram negative pathogens*	

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	such as Actinobacillus, Pasteurella, Mannheimia, Histophilus and Gram +ve major veterinary pathogens (Staphylococcus, Streptococcaceae, Trueperella, Listeria, Erysipelothrix etc), first line beta-lactams are natural penicillins (i. e penicillin G, penicillin V, penethamate e*tc). i. e. narrow-spectrum penicillins, not aminopenicillins (e. g. amoxicillin).* Additionally, for *Staphylococcus*, International Institutions accredited for the standardisation of antimicrobial susceptibility testing in both humans and animals (CLSI and EUCAST), based on scientific evidence and with a prudent-use perspective, clearly points towards recommendations to test *Staphylococcus* susceptibility towards benzylpenicillin (prototype of narrow-spectrum penicillins) and cefoxitin only. When susceptible to benzylpenicillin, they clearly suggest using narrow-spectrum penicillins as first choice. Isolates that test as resistant to benzylpenicillin but susceptible to cefoxitin are susceptible to and can be still successfully treated with-penicillinase-stable beta-lactams: the isoxazolylpenicillins (e.g. VMP cloxacillin, dicloxacillin), nafcillin, β-lactamase inhibitor combinations (e. g. amoxicillin-clavulanic acid), oral and parenteral cephems (e. g. first generation cephalosporins like cephalexin, cefazolin, cefalotin). Cefoxitin-resistant *Staphylococcus* isolates are thus classified as resistant to all beta-lactam antibiotics and beta-lactam combinations, except for some antibiotics not authorized in Veterinary Medicine and reserved for human use only. This approach meets the requirements and principles for a prudent use of beta-lactams and helps limiting to particular cases only the need of using potentiated aminopenicillins and especially 3rd & 4th generation cephalosporins for treating Staphylococcus clinical disease in animals. In conclusion, besides a necessary change in the current new AMEG	

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	classification, for aminopenicillins, we kindly suggest to specify that there is need to reduce the overall use, especially antimicrobials given via the oral route, AND avoid their unnecessary, inappropriate (from the pharmacological and clinical point of view), and non-prudent use. Indeed, we believe that recommendations on risk management are the essence of this kind of documents, irrespective of how thorough the review on the topic may be. Conclusions should be in-line with the main points reported in the document. b. Comments on the "new AMEG Classification" ("Answer to the request from the European Commission for updating the scientific advice on the impact on public health and animal health of the use of antibiotics in animals - Categorisation of antimicrobials") i. Comments on classification of aminopenicillins We acknowledge that in the Table on aminopenicillins, a note has been added: "In case accumulating evidence from future scientific research indicates that veterinary use of aminopenicillins poses an added threat to public health due to animal-to-human resistance transfer, it could then be considered if a distinction in the categorisation should be made between straight aminopenicillins and narrow-spectrum penicillins" However, even from the Concept paper for the development of a reflection paper on the use of extended-spectrum penicillins in animals (2015) EMA had already acknowledged (see lines 60 -) the following: "Veterinary authorised Extended-Spectrum aminopenicillins and their combinations with beta-lactamase inhibitors are classified as Category 2 by EMA/AMEG pending a final decision on the category. Veterinary use of aminopenicillins might have the ability to facilitate the spread of antimicrobial resistance	

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	similarly to 3rd- and 4th-generation cephalosporins, and therefore further risk profiling is needed (EMA, 2014)." https://www.ema.europa.eu/documents/scientific-guideline/draft-concept-paper-development-reflection-paper-use-extended-spectrum-penicillins-animals-european_en.pdf Naturally, it would be very welcome if additional evidence with controlled experiments could investigate the capacity of aminopenicillins to select for and promote the spread (or maintain the presence of) of Extended-Spectrum Cephalosporin resistance in groups of food-producing animals. However, even before bringing additional data and funding for further investigations, the EU could use and analyse available EU-wide data already available: a. ESVAC data and b. EU AMR monitoring data These data come from activities harmonised at the EU level, and could be better explored in order to bring useful information on ecological and epidemiological associations. Indeed, every other year the JIACRA reports include the results of a logistic regression approach to explore the association between antimicrobial *consumption* in animals and *resistance prevalence in animals and humans.* It would be important to check whether there is a statistical association between the level of use of aminopenicillins and resistance to 3rd & 4th generation cephalosporins in food-producing animals. In other words, if there is an association between the amount of broad-spectrum penicillins usage and prevalence of resistance towards 3rd & 4th generation cephalosporins. See pag. 42, Ch. 6.2. of the 2nd JIACRA Report: The analysis is limited to "the use of 3-4th gen cephs and the level of resistance to 3-4th gen cephalosporins." The use in food-producing animals	

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	of 3rd & 4th generation cephalosporins (with a range depending on the different MS farmers' attitude to their usage) in EU is invariably lower than for other antibiotics: however, when we investigate prevalence of Extended-spectrum Cephalosporin (ESC) – resistance in intestinal *E. coli* in animal productions from several EU countries, we find 70-80% herd prevalence. Also this is the case in poultry, where these last resort antimicrobials have never been licensed. Thus, here we suggest retrospectively and prospectively extending the epidemiological analysis of data used for the JIACRA reports for better assessing this issue. ii. Comments on classification of macrolides Macrolides should be included in Category B, not in Category C. We are very concerned about the AMEG proposal of placing macrolides into Category C ("Caution"), especially since the WHO document on Critically Important Antimicrobials clearly places macrolides among HPCIAs (https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia/en/), and further suggests in the WHO food animal guideline (https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia_guidelines/en/) that HPCIAs should not be used in healthy food animals for prophylaxis/metaphylaxis. The proposal of placing macrolides in AMEG Category C may be at risk of offering arguments for not reducing the usage of macrolides via the oral route in animal productions (especially in pigs and poultry), which negatively impacts resistance towards macrolides in Campylobacter and other zoonotic Gram negative (*Salmonella*), Gram-positive (LA-MRSA) and opportunistic pathogens (*E. coli*) to humans. The AMEG report states that if certain genes encoding acquired macrolide resistance (e.g. *cfr*, *erm* genes) are identified with increased prevalence in food animal isolates, then AMEG will reconsider the classification.	

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	Unfortunately, these genes have already been identified in EU food animal bacterial isolates (see joint EFSA-ECDC EU Annual Reports on AMR). Additionally, *erm*-mediated macrolide resistance is already a very common feature in Gram-positive zoonotic pathogens such as LA-MRSA CC398, CC1, CC97, while is emerging in *Campylobacter jejuni* and *C. coli*. Also, high-level macrolide (azithromycin) resistance mediated by *mph* genes (not mentioned in the AMEG Report) has already been reported in the EU in *E. coli* and *Salmonella*. In Italy, macrolide resistance in *C. jejuni* from broiler chicken is at 8% (EU Summary Report AMR 2016). Ciprofloxacin-resistant *Campylobacter* is already common and highly prevalent throughout Europe and if macrolide resistance spread then this would further compromise public health. Macrolide resistance and it is also an emerging problem in *Salmonella* spp. (and *E. coli*) from animal productions in EU. Azithromycin (registered for human use only) has been chosen as the prototype macrolide antibiotic by the harmonised AMR monitoring according to the EU legislation. In some cases, azithromycin resistance in *Salmonella spp*. in broilers reaches 6% (Portugal) and 8% (Germany) in 2016. In indicator *E. coli*, it exceeds 10% in some EU countries (See Table 17, p. 81 and Table 45, p. 179, respectively, of the EU Summary Report AMR 2016). (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5182). In the new AMEG document, it reads: "Category C ("Caution") was therefore added in this report as an intermediate category. This category includes antimicrobial classes listed in different categories by WHO, including macrolides, which are listed by WHO as a 'highly prioritised CIA'. There are in general alternatives in human medicine in the EU but there are few alternatives in veterinary medicine for certain indications." However,	

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	the only argument brought for "few alternatives" for macrolides in veterinary medicine is "*Lawsonia intracellularis* infection". We are concerned about this comment as well as about the risk that macrolides may be used in subtherapeutic or even therapeutic dosages by oral route for the "prevention", "metaphylaxis" or "treatment" of *L. intracellularis* "infections" even when there is no sign of disease (i. e. chronic or acute ileitis). Instead of in case of "infection", macrolides should be used only against the "disease" caused by *L. intracellularis*, i.e. ileitis, and a more prudent approach would be to use macrolides on an individual basis (i. e. by injection) only in animals showing signs of clinical disease. This argument should be acknowledged by the AMEG document. Indeed, there is the risk that the diagnosis of "infection" by *L. intracellularis* may be obtained by serology or PCR only (the majority of holdings are likely to test positive in several EU countries...), irrespective of any clinical or pathological finding. Unfortunately, the claim for *L. intracellularis* "infection", appears to be the main argument to keep on administering courses of macrolides to all animals within a group. Additionally, in general and in the specific case of macrolides, the document does not take into account that valid alternatives to antibiotic "prevention, metaphylaxis or even treatment" with macrolides are available for porcine ileitis, such as attenuated and inactivated vaccines. It is worth noting that vaccines for immunization against *L. intracellularis* infection are available on the EU market (e. g. Enterisol Ileitis Vet), and more are currently under regulatory procedures. See earlier and recent literature: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5846845/ Also, tetracyclines can be used for *L. intracellularis* disease in pigs. Macrolides are not an essential treatment in Europe for *L. intracellularis*	

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	disease. In conclusion, we believe that for macrolides it would be necessary to rethink their categorisation: reasons for restricting oral administration to groups of animals only to specific cases, and with specific diagnosis of bacterial disease, should be taken into consideration by the European Commission. Additional References: The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food" (2015: fattening pigs and veal calves, 2016 broilers and turkeys). https://www.efsa.europa.eu/en/efsajournal/pub/4694 https://www.efsa.europa.eu/en/efsajournal/pub/5182 *Erm* genes and LA-MRSA Belgium https://www.ncbi.nlm.nih.gov/pubmed/26350798 Italy https://www.sciencedirect.com/science/article/pii/S0378113509005239?via%3Dihub https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4556339/ https://aem.asm.org/content/82/3/816 *Erm* genes in C. jejuni https://www.ncbi.nlm.nih.gov/pubmed/29632015 - Chen JC, Tagg KA, Joung YJ, Bennett C, Francois Watkins L, Eikmeier D, Folster JP. Report of erm(B)(+) Campylobacter jejuni in	

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	the United States. Antimicrob Agents Chemother. 2018 May 25;62(6). pii: e02615-17. doi: 10.1128/AAC.02615-17. Print 2018 Jun. - Florez-Cuadrado D, Ugarte-Ruiz M, Quesada A, Palomo G, Domínguez L, Porrero MC. Description of an erm(B)-carrying Campylobacter coli isolate in Europe. J Antimicrob Chemother. 2016 Mar;71(3):841-3. doi: 10.1093/jac/dkv383. https://academic.oup.com/jac/article/71/3/841/2363740	
4	Reviewed the draft paper "Reflection paper on the use of aminopenicillins and their beta-lactamase inhibitor combinations in animals in the European Union: development of resistance and impact on human and animal health" (reflection-paper-use-aminopenicillins-their-beta-lactamase-inhibitor-combinations-animals-european_en.pdf), found on the internet. "The stated objective of this document is to review available information on the use of these substances in veterinary and in human medicine in the EU, the influence that veterinary use in particular has on the emergence of AMR and its potential impact on human and animal health. The document provides information for risk profiling, as recommended by the Antimicrobial Advice ad hoc Expert Group (AMEG), which will allow these substances to be placed within the AMEG's categorization." (Lines 218-223) The reflection paper (RP) has several positive features by providing an update on usage of aminopenicillins in Veterinary Medicine for target veterinary bacterial pathogens, mechanisms of resistance, as well as risks for human health arising from usage of such antibiotics in animals. The RP does a particularly good job on describing the general drug characteristics of aminopenicillins. Reviewing any beta-lactam drug class is challenging given the volume of scientific literature available and the complexity of the	Not agreed. The JIACRA report is mentioned in order to highlight that quantifying the transmission of resistance is complex. Reports that analyze the relationship between antimicrobial consumption in animals and humans and the occurrence of resistance are scarce. It clearly states that: " Although the report did not investigate the consumption of aminopenicillins in food-animal species and aminopenicillin or other resistance in human bacteria, the results show that the epidemiology of resistance is complex and several factors other than the amount of antimicrobials consumed may influence the level of resistance." And "JIACRA II (ECDC/EFSA/EMA, 2017) pointed out associations between fluoroquinolone consumption in food-animals and fluoroquinolone resistance in zoonotic bacteria of humans while such association was not detected for 3rd and 4th-generation cephalosporins. While this report did not

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	issues. This RP provides a useful reflection on general drug characteristics, that is both easy to read and a useful update. The major faults of the RP, in the current version, concern the Discussion and Conclusions that are unfounded in scientific evidence, and not consistent with the evidence presented as well as other knowledge about aminopenicillins (please see below). In general, the reflection paper is not balanced and should include points of raising awareness to the possible misuse of aminopenicillins in an animal Health and a One Health perspective. Lack of balance of the RP is further evident by not examining the international scientific literature for evidence of the risk of transfer of relevant resistance from animals to humans. Also, there are examples where the RP is not consistent (or goes beyond) with the mandate given by the CVMP. Some examples include: - JIACRA report is summarized in a couple of places in the RP. It is unclear as to why the JIACRA report is mentioned since it did not specifically focus on aminopenicillins. Mentioning the JIACRA report findings on fluoroquinolones and 3rd & 4th generation cephalosporins is outside the mandate given by the CVMP. A useful conclusion could be to recommend that JIACRA focus on aminopenicillins in relation to resistance to 3rd & 4th generation cephalosporins. - "…, based on the extent of use of these drugs in humans, the major resistance selection pressure in human pathogens caused by aminopenicillin use in European countries can be considered to be due to human consumption of these or other related beta-lactam drugs." (Lines 1543-1546). It is unclear how statements like this are consistent with the mandate given by the CVMP. The mandate was specifically for risk profiling of veterinary use of	estimate the association of aminopenicillin consumption and antimicrobial resistance, it confirmed the positive association between AMC and AMR in both humans and food-producing animals highlighting the need for prudent use and to reduce the AMC in both sectors." Therefore, we decide to keep the sections on the JIACRA reports, although the reports does not analyze aminopenicillins. This is also clearly stated in the RP. The mandate of the CVMP was to review available information on the use of these substances in veterinary and in human medicine in the EU, the influence that veterinary use in particular has on the emergence of AMR and its potential impact on human and animal health. For this purpose, it is important to also take into account the human use and its effect on human medicine as risks are not absolute and should be seen in a One Health context. It is important to note that the use of aminopenicillins in humans is twice the use of animals and that the percentage of resistance in certain human pathogens is very high. Antimicrobials should be used prudently in human as well as in animal medicine. Generally, there is no lack of prudent use guidelines in veterinary or human medicine. What is lacking in some countries is the implementation of these guidelines. There is still misuse of antibiotics in humans as well as in veterinary medicine. This RP is not on the stratification of the aminopenicillins,

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	aminopenicillins on human and animal health. It was not about human medical use of aminopenicillins on human health. Furthermore, the following comments are also relevant to this statement: ○ What methodology was used to assess EU human aminopenicillin data in relation to EU aminopenicillin resistance patterns? How is this methodology transparent in the RP? Does this specifically have an impact on human clinical isolates or the general human population? It is worth noting that the majority of the general population does not consume aminopenicillin on a regular basis. The main use of aminopenicillins in human medicine is for vulnerable populations (e.g. infections, elderly, pediatrics, cancer patients). However, the major use in food animals is healthy animals as either prophylaxis or metaphylaxis. ○ Also, this statement does not take into consideration that if aminopenicillins are used "prudently" in human medicine then it may not contribute to EU aminopenicillin resistance patterns. ○ If the authors of the RP feel this reflects 'common knowledge' that both high consumption of aminopenicillins in human populations contribute more to resistance patterns in human isolates then this same 'common knowledge' is not applied consistently in this RP to veterinary use of aminopenicillins. Specifically, there is high consumption of aminopenicillin use (without inhibitor) in animals (especially food animals), then the same principle should apply that this is a major resistance selection pressure in animal isolates related to the food-chain.	but a review of the literature and data available. The stratification has been addressed in the AMEG and part on the AMEG categorization have therefore been removed from this RP. Prudent use of aminopenicillins in veterinary medicine is important and this is mentioned more clearly now in the RP. In addition, it is mentioned that narrow spectrum penicillins with a lower risk of selection for resistant bacteria should be used for first line treatment wherever possible. This sentence has been deleted: "Currently there is no evidence indicating that the use of aminopenicillins in animals would be associated with aminopenicillin or other resistance in human bacteria." It is stated that: "It is clear that resistant organisms are transferred between animals and humans, but the direction and magnitude of transfer is often difficult to prove or quantify, except for the major food-borne zoonotic pathogens" "Aminopenicillin use in animals may select resistance in zoonotic or other bacteria of animal origin that can further be transferred to humans, but based on the extent of use of these drugs in humans, it *seems probable that the major resistance selection pressure in human pathogens caused by aminopenicillin use in European countries is due to human consumption of these or other related beta-lactam drugs*."

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	Discussion: "All these factors should be taken into account for the AMEG's categorisation, which is currently under review. It is suggested that the AMEG could give consideration to a further stratification of the categorisation to allow a distinction in the ranking between those substances currently in Category 2 (fluoroquinolones, 3rd- and 4th-generation cephalosporins and colistin, for which there are fewer alternatives) and the amoxicillin-clavulanate combinations, and between the latter and the straight aminopenicillins. Amoxicillin-clavulanate has a wider spectrum and thus it is likely that it has higher chance to select multidrug resistant organisms compared to aminopenicillin alone." - It is worth a paragraph in the RP stating the pros and cons of stratification of aminopenicillins. Stratification suggests a lower risk for aminopenicillins alone, for which the case has not been made in this RP. The idea of stratification has been suggested previously (Burch & Sperling 2018 Amoxicillin—current use in swine medicine. *J vet Pharmacol Therap.* 41:356–368.). - Also, there are relevant negative effects for pigs if aminopenicillins alone are considered low risk and used commonly. o "Oral administration of amoxicillin produced very low peak plasma concentrations in pigs, ranging from 0.2 to 3.1 mg/L depending on dose (10-23 mg/kg), and on whether the drug was given as oral bolus, or in feed or drinking water (Agersø and Friis, 1998a; Agersø et al., 1998; Godoy et al., 2011)." (Lines 352-354) o "Aminopenicillin resistance in clinical *E. coli* isolates from	and "Commensal bacteria in animals, such as Enterobacteriaceae, may act as a reservoir for resistant bacteria or resistance genes that may be transferred to bacteria in humans; however, the high extent of aminopenicillin use in humans itself provides a selection pressure for resistance in the human microbiota. *Recent studies have indicated that most community-acquired ESBL/pAmpC-E. coli carriage was attributed to human-to-human transmission. Therefore, the significance to public health of additional aminopenicillin resistance for commensal ESBL/pAmpC Enterobacteriaceae transferred from animals is likely to be relatively low regarding the already high level of resistance in humans*." We agree that the consumption of amoxy+clav should remain low. We added a sentence to the recommendations: *"The consumption of amoxicillin-clavunate should remain low particulary in food-producing animals and used with caution in all animal species. These substances should only be used when there is no available substance from category D that would be clinically effective."*

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	pigs is very frequent. For example in 2015, the level of amoxicillin resistance was reported as 55% in France (Anses, 2016), nearly 40% (ampicillin) in UK (UK-VARSS, 2015), and close to 40% in Sweden (Swedres-Svarm, 2016) in *E.coli*. The respective figure for amoxicillin-clavulanic acid resistance in France was 18%, while in the UK it was less than 10%." (Lines 938-942) ○ "The treatment of pigs with amoxicillin or ceftiofur during the rearing period was linked to emergence of cephalosporin resistant *E. coli*, but these bacteria were no longer present by the time of finishing (Cameron-Veas et al., 2016; Cameron-Veas et al., 2015)." (Lines 1139-1142) It is unclear as to the reason/s in this RP for suggestion stratification of aminopenicillins, apart from the fact that aminopenicillins (with inhibitor) have a broader bacterial spectrum of action. No evidence has been presented that these aminopenicillin combination products do indeed exert a different selection pressure for multi-drug resistant bacteria, more so compared to aminopenicillins alone. A Danish study by Cavaco *et al*. (2008) compared gut *E. coli* selection pressure using injections of amoxicillin, ceftiofur and cefquinome, given IM at 15, 3 and 3 mg/kg bodyweight, respectively, for three consecutive days. The pigs were inoculated intragastrically with 1010 colony-forming units (CFU) of a nalidixic acid (NA)-resistant mutant derived from a CTX-M1 producing porcine *E. coli*. The total coliforms and CTX-resistant coliforms were counted, and the total resistant coliforms to NA and CTX, the original isolate, were also counted. Significantly higher counts of CTX-resistant coliforms were observed in the three treatment groups than in the controls over the 25-day trial period. This demonstrates that aminopenicillins alone can select for persistence for the same bacterial resistance as 3rd & 4th generation cephalosporins	

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	(Cavaco, L. M., Abatih, E., Aarestrup, F. M., & Guardabassi, L. 2008 Selection and persistence of CTX-M-producing Escherichia coli in the intestinal flora of pigs treated with amoxicillin, ceftiofur or cefquinome. *Antimicrobial Agents and Chemotherapy*, *52*(10), 3612–3616. https://doi.org/10.1128/AAC.00354-08). Also, it is worth pointing out that the EU epidemiological evidence points to a spread of extended-spectrum beta-lactam resistance in Enterobacteriaceae in animals, especially resistance to 3rd & 4th generation cephalosporins (herd-prevalence of ESBL/AmpC-producing *E. coli* in the intestines of food-producing animals is around 50-90% in many EU countries). See "*The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food*" (EUSR AMR 2015: fattening pigs and veal calves, EUSR AMR 2016 broilers and turkeys). https://www.efsa.europa.eu/en/efsajournal/pub/4694 (EUSR AMR 2015) https://www.efsa.europa.eu/en/efsajournal/pub/5182 (EUSR AMR 2016). This in spite of the fact that aminopenicillins alone are consumed more in the EU animal populations than aminopenicillin combination products (with inhibitor). - Also, statements in this RP suggest that aminopenicillins alone can select for the same type of resistant bacteria as aminopenicillin combinations (with inhibitor), including: ○ "It was later observed that elevated expression of these enzymes can be due to reversible induction, i.e. elevated expression persists as long as the inducer is present. Many beta lactams like benzylpenicillin, ampicillin, amoxicillin, and cephalosporins (e.g. cefazolin and cephalotin) are strong inducers of AmpC enzymes. Also clavulanic-acid, although having little inhibitory effect on AmpC enzymes on its own, can paradoxically increase these enzymes in an	

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	inducible bacteria (Jacoby, 2009).” (Lines 511-516) ○ “In general, in 1961 transferable beta-lactamase resistance was an unknown phenomenon while forty years later already 200 different beta-lactamases had been identified. Since then, evolution has escalated: today more than 1300 different beta-lactamase variants exist. The emergence of ESBL, AmpC and carbapenemases just within the two last decades has been rapid.” (Lines 525-528) ○ “Ampicillin or amoxicillin-clavulanic acid susceptibility results do not provide information on whether the bacterial isolate in question produces broad spectrum beta lactamases (ESBL/AmpC/CPE). Therefore it is vital that testing panels in veterinary laboratories include antimicrobials that facilitate the recognition of bacterial isolates that may have reduced susceptibility to 3rd-generation cephalosporins or carbapenems even though testing would not be necessary for clinical purposes.” (Lines 610-615) ○ “The treatment of pigs with amoxicillin or ceftiofur during the rearing period was linked to emergence of cephalosporin resistant *E. coli*, but these bacteria were no longer present by the time of finishing (Cameron-Veas et al., 2016; Cameron-Veas et al., 2015).” (Lines 1139-1142) ○ “In addition to cephalosporin resistance, amoxicillin and ampicillin are capable of co-selection of multi drug resistance in *E. coli* (Bibbal et al., 2009; Dheilly et al., 2012; Persoons et al., 2011). In chickens, a two day course of amoxicillin either with a full dose, or 75% of the full dose, selected resistant isolates (van der Horst et al.,	

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	2013). This was observed for tetracycline and fluoroquinolones as well, but amoxicillin seemed to have the strongest effect on selection of resistance after a two week follow up-period, although resistance declined in all treatment groups during this period. Aminopenicillins may have a role in maintaining and selecting AmpC- and ESBL-carrying *E. coli* once introduced to a herd, even without use of cephalosporins, as was observed in poultry farms in Denmark (Agersø et al., 2014)." (Lines 1143-1151) ○ "In a Belgian study ampicillin resistance in porcine *E. coli* was associated with the use of amoxicillin, ceftiofur or enrofloxacin. Regardless of the mode of administration (intramuscular route or oral route under fed or fasting conditions), a 7-day course of ampicillin increased the proportion of the ampicillin resistant faecal *E. coli* population with simultaneous increase of TEM coding beta-lactamase genes (Bibbal et al., 2009)." (Lines 1125-1130) ○ "Possibly due to fact that amoxicillin clavulanic-acid is far less used than aminopenicillins in food producing animals, there is lack of data on how this combination selects resistance in animals." (Lines 1164-1165) ○ "Although the major selection force for extended spectrum cephalosporin resistance is considered to be the use of cephalosporins and fluoroquinolones, aminopenicillins, especially inhibitor combinations, may co-select such resistance as can several other antimicrobials if the organism harbours the determinants conferring resistance to cephalosporins and fluoroquinolones in addition to aminopenicillin resistance." (Lines 1485-1489)	

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	○ "However, aminopenicillins without inhibitors are also able to co-select extended spectrum beta-lactam or multi-drug resistance due to simultaneous carriage of several resistance genes by many bacterial isolates." (Lines 1506-1508) Based on the information presented in this RP then a reasonable argument can be made for association between consumption of aminopenicillins in animal or human populations and the incidence of aminopenicillin-resistant bacteria. "Measured in mg/PCU, penicillins [extended spectrum penicillins (ampicillin, amoxicillin), beta lactamase sensitive penicillin (benzyl penicillin, penethamate, phenoxymethylpenicillin) and beta lactamase-resistant penicillins (cloxacillin, dicloxacillin)] were the second most sold antimicrobial class in food animal species in the EU in 2015 and accounted for 25% of the total antimicrobial sales (EMA/ESVAC, 2017)." (Lines 636-640) - "Extended spectrum penicillins (ampicillin, amoxicillin, and their inhibitor combinations) made up the major proportion (88%, 30.0 mg/PCU) of the total use of penicillins (Figure 2), although wide variation between the member states was observed. There were only six European countries (Denmark, Finland. Iceland, Luxembourg, Norway, Sweden) in which beta lactamase sensitive penicillins (benzyl penicillin, penethamate, phenoxymethylpenicillin) contributed more than half of the total beta-lactam sales, while in 23 out of 30 countries, amoxicillin and ampicillin consumption contributed more than half of the total penicillin sales. Aminopenicillins and their inhibitor combinations formed a very limited fraction of the total sales of aminopenicillins both at the European level (1%, 0.3 mg/PCU) and by country (Figure 2 and	

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	Figure 3).” (Lines 641-649) - This is further complicated by the fact that aminopenicillins alone are given more indications in SPCs compared to combination products. o “In addition to the treatment of infections in various organs, metaphylactic or prophylactic indications are included in SPCs. For products containing amoxicillin-clavulanic acid, the spectrum of different indications is narrower compared to ampicillin and amoxicillin products, but is still wide.” (Lines 659-662) Thus, aminopenicillins alone in animals are known to have significantly higher consumption in the EU compared to aminopenicillin combination products (with inhibitor), as well as broader indications that include mass medications. This difference in EU consumption is later in the text of this RP reflected in EU surveillance data on resistant bacteria, noting the following: - “Aminopenicillin resistance in clinical *E. coli* isolates from pigs is very frequent. For example in 2015, the level of amoxicillin resistance was reported as 55% in France (Anses, 2016), nearly 40% (ampicillin) in UK (UK-VARSS, 2015), and close to 40% in Sweden (Swedres-Svarm, 2016) in *E.coli*. The respective figure for amoxicillin-clavulanic acid resistance in France was 18%, while in the UK it was less than 10%.” (Lines 938-942) - “For Pasteurellaceae, aminopenicillin resistance is most frequently reported in *Actinobacillus pleuropneumoniae.* An Italian study reported an increasing trend in beta-lactam resistance for this species from 1994 - 2009 (Vanni et al., 2012). The same study also reported high and variable resistance figures for different beta-lactams: 69% for ampicillin, 83% for amoxicillin and 9% for amoxicillin-clavulanic acid.” (Lines 955-959)	

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	- "In the UK, aminopenicillin resistance was observed in 9 - 17.6% of *A. pleuropneumoniae* isolates, depending on animal population. None was resistant to amoxicillin-clavulanic acid (UK-VARSS, 2015). In France, only 2% were resistant to aminopenicillins, but none to amoxicillin-clavulanic acid or ceftiofur (Anses, 2016). *Pasteurella multocida* is generally susceptible to aminopenicillins (El Garch et al., 2016; (Anses, 2016) or resistance rate is low (UK-VARSS, 2015)." (Lines 961-965) - "Aminopenicillin non-susceptibility is high among cattle clinical *E. coli* ranging from 26% to 85% in different EU countries, depending on year and cattle population. Resistance rates for amoxicillin clavulanic acid are lower than for ampicillin or amoxicillin." Lines (972-974) - "According to French and UK surveillance, aminopenicillin resistance in *E. coli* from poultry infections is very common, up to 50% depending on animal age or species in question. Approximately 10% resistance was reported to amoxicillin-clavulanic in *E. coli*, but only a few percent resistance for 3rd cephalosporins (Anses, 2016; UK-VARSS, 2015). Penicillin/aminopenicillin resistance in *Staphylococcus aureus* from poultry is 0 – 13%, being highest for *S. aureus* isolates in turkeys in France (Anses, 2016)." (Lines 998-1003) Thus, the stratification of aminopenicillins is not justified in the RP due to issues identified: - Stratification will lead to the same or higher consumption of aminopenicillins alone that also contributes to the same high EU resistant rates in animals or higher. - Evidence suggests that aminopenicillins alone can select for the	

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	same resistant bacteria as aminopenicillin combinations (with inhibitor). Evidence to the contrary has not been presented in this RP. For example, currently 3rd & 4th generation cepahlosporins are restricted in the EU for food animals. The benefits of these initiatives will be counter-acted by the high use of aminopencillins that can select the same resistant genes. - Stratification of aminopenicillins is not done in the WHO classification and since aminopenicillins are WHO CIAs then they are not recommended by WHO for mass medication purposes or first-choice treatments. - Aminopenicllins alone are more likely to be given both orally and for mass medications. This creates the highest risk for resistance selection and transfer and contrary to statements made in this RP: o "Aminopenicillins are capable of selecting both aminopenicillin resistance and also resistance to other antimicrobials in the gut microbiota of dogs (Edlund and Nord, 2000; Grønvold et al., 2010). In a mouse model, oral *versus* injectable (i.v.) ampicillin significantly resulted in more ampicillin-resistant strains and resistance genes (*bla*CMY-2) in the gut microbiota (*E. coli*) (Zhang et al., 2013)." (Lines 1152-1155) o "Of importance, where oral antimicrobial treatments are given to large groups, the resistome in faecal indicator bacteria and pathogens in livestock is much more vulnerable to selection pressure compared to animals kept individually, or in small groups, and if injectable treatment is given (Catry et al., 2016). Therefore interventions to minimize the effect of oral administration of antimicrobials on AMR in the commensal bacteria and target pathogens	

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	should be considered." (Lines 1155-1160) "In case accumulating evidence from future scientific research indicates that veterinary use of aminopenicillins poses an added threat to public health due to animal-to-human resistance transfer, it could then be considered if a distinction in the categorisation should be made between straight aminopenicillins and narrow-spectrum penicillins" (Lines 174-177) - However, even from the Concept paper for the development of a reflection paper on the use of extended-spectrum penicillins in animals (2015) EMA had already acknowledged (see lines 60 -) the following: "Veterinary authorised Extended-Spectrum aminopenicillins and their combinations with beta-lactamase inhibitors are classified as Category 2 by EMA/AMEG pending a final decision on the category. Veterinary use of aminopenicillins might have the ability to facilitate the spread of antimicrobial resistance similarly to 3rd- and 4th-generation cephalosporins, and therefore further risk profiling is needed (EMA, 2014)." - It is further unclear as to why this statement about accumulating evidence is stated in the RP. In the same RP it is stated that "Considering that aminopenicillin resistance is at a very high level in some organisms and that aminopenicillins have been extensively used for decades both in animals and humans, it is currently impossible to estimate to what extent the use of these substances in animals, could create negative health consequences to humans at the population level." (Lines 123-126). However, it is not clearly stated in the RP as to what evidence is needed. Without specifying what is needed then any additional evidence is likely to also be deemed as 'impossible' to assess the impact of animal-associated	

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	aminopenicillin-resistance on public health. "…whilst the risk for resistance transfer by consumption of food of animal origin is considered low, especially if good food hygiene practices are followed." (Lines 1522-1523) - In the EU, there are thousands of cases annually of zoonosis from food of animal origin (e.g. Salmonella, Campylobacter, E. coli, etc …). It is presumed that good hygiene practices are followed OR this RP has not presented evidence to the contrary. Exactly what good hygiene practices should be followed that contribute to "low" risk for aminopenicillin transfer from food of animal origin that are not being followed in cases of zoonosis from food of animal origin? Conclusions: 1) "Currently there is no evidence indicating that the use of aminopenicillins in animals would be associated with aminopenicillin or other resistance in human bacteria. More research is needed to explore AMC in food-producing animals and AMR in humans." (Lines 1510-1512) - However, "It is clear that resistant organisms are transferred between animals and humans, but the direction and magnitude of transfer is often difficult to prove or quantify, except for the major food-borne zoonotic pathogens." (Lines 1513-1515) - Thus, IF "it is clear that resistant organisms are transferred between animals and humans …" then it is not logical to conclude that "Currently there is no evidence indicating that the use of aminopenicillins in animals would be associated with aminopenicillin	

Stakeholder no.	General comments	Outcome (if applicable)
	or other resistance in human bacteria." 2) "The significance to public health of additional aminopenicillin resistance transferred from animals is considered to be low." (Lines 1582-1583) - It is unclear as to how resistance transfer from food of animal origin was assessed as "low". What methodology was used and why is this not transparent in the RP? - No scientific evidence or summary of evidence is provided to support this conclusion of resistance transfer from food of animal origin to be assessed as "low". Both these conclusions are not based on a logical assessment of the information presented. Although, not well explained in this RP the use of aminopenicillins in animals would select for aminopenicillin-resistant bacteria that could be transferred (e.g. direct contact, exposure to food if animal origin, consumption of food of animal origin) to humans via two major mechanisms, including 1) resistant bacterial clones (e.g. zoonotic bacteria, microflora); 2) transmissible resistance via mobile genetic elements from animal adapted bacterial clones to human adapted bacterial clones. On these issues, the RP states the following: - "Considering that aminopenicillin resistance is at a very high level in some organisms and that aminopenicillins have been extensively used for decades both in animals and humans, it is currently impossible to estimate to what extent the use of these substances in animals, could create negative health consequences to humans at the population level." (Lines 123-126)	

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	- "Due to the complexity of AMR epidemiology and the near ubiquity of some aminopenicillin resistance determinants, the direction of transfer – whether gene or resistant isolate - may be difficult, if not impossible, to ascertain, except for major food-borne zoonotic pathogens like *Salmonella* spp., and certain LA-MRSA clones." (Lines 1492-1495) If it is 'impossible' to assess the impact of animal-associated aminopenicillin-resistance on human populations then it is not logical to conclude that "The significance to public health of additional aminopenicillin resistance transferred from animals is considered to be low." (Lines 1582-1583) The only logical conclusion is that it is not possible to conclude on the significance to public health of aminopenicillin resistance transferred from animals. It is worth noting that the majority of beta-lactam resistant genes are present commonly on mobile genetic elements, which is associated with higher risks of resistance transfer. Despite the 'impossibility' to conclude on the issue of transmissible resistance between animal and human bacteria clones, this RP states the following: - "The gene *bla*CMY-2 confers resistance to aminopenicillins, extended-spectrum cephalosporins and the inhibitor clavulanate. In a Norwegian study, *E. coli* resistant to extended-spectrum cephalosporins recovered from retail chicken meat and carrying an IncK plasmid with the *bla*CMY-2 gene (N=17) were compared by whole genome sequencing with human clinical *E. coli* isolates (N=29) which also carried an IncK plasmid bearing the *bla*CMY-2 gene. The plasmid in all 29 human *E. coli* isolates was highly similar to that present in the poultry isolates (Berg et al., 2017)." (Lines 1352-1357)	

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	- "Carriage of SHV beta-lactamases by a number of different plasmid types has facilitated widespread dissemination into diverse ecological niches including surface waters, food-producing animals and food such as retail meat derived from animals (Liakopoulos et al., 2016)." (Lines 1376-1378) - "The widespread dissemination of *S*. Typhimurium DT104 has also resulted in dissemination of the integron and *bla*CARB-2 resistance gene it usually carries. Food-borne zoonotic transmission of *S*. Typhimurium DT104 from animals to man (as well as transmission through direct contact with animals), provides a means of transmission of resistance between animals and man." (Lines 1400-1404) - "The ROB-1 beta-lactamase gene, belonging to class A-beta-lactamases, has been described in *A. pleuropneumoniae* (Juteau et al., 1991) The same gene has been detected in other bacterial species belonging to the family Pasteurellaceae isolated from animals and humans (Livrelli et al., 1991) and is considered to be of animal pathogen origin (Medeiros et al., 1986). The plasmid encoded beta lactamase ROB-1, detected in *A. pleuropneumoniae* isolates from pigs, was also detected in the human meningitis pathogen *H. influenzae* Type b in the USA (Medeiros et al., 1986), although the majority of beta-lactam resistance in *H. influenzae* was related to the presence of the beta-lactamase TEM-1, which is extremely widespread in bacteria from both human and animal bacteria. The plasmids carrying ROB-1 were found to be very similar in both *A. pleuropneumoniae* and *H. influenzae* Type b suggesting transfer between these bacterial species. The available epidemiological information did not indicate direct contact with pigs in human cases of meningitis *H. influenzae* Type b carrying ROB-1	

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	(Medeiros et al., 1986).” (Lines 1405-1416) - “During the last 15 years, ESBL producing TEM, SHV and CTX-M or AmpC-producing, CMY-carrying Enterobacteriaceae (mainly *E. coli* and *Salmonella* spp.) have also been increasingly reported in food-producing animals and food (EFSA BIOHAZ Panel, 2011). The distribution of different ESBL-enzymes is similar in bacteria of animal and human origin.” (Lines 1428-1432) - “Within recent years, also bacteria carrying acquired carbapenemases, such as VIM-1 producing *E. coli* and *Salmonella* spp., OXA-23 and NDM-1 positive *Acinetobacter* spp. have emerged in pigs, cattle and poultry (Guerra et al., 2014).” (Lines 1451-1453) - “The same or similar resistance genes have been isolated in bacteria of human and animal origin, and molecular studies suggest that resistance gene transmission or transmission of bacteria with resistance to aminopenicillins occurs between bacteria of animal, human, food or environmental origin (Madec et al., 2017).” (Lines 1489-1492) - “Nevertheless, the existence of these common resistance determinants in animal bacteria has raised concern about food-producing animal reservoirs for antimicrobial resistance (EFSA BIOHAZ Panel, 2011), which is of major concern for zoonotic pathogens causing illness in humans (*Salmonella* and *Campylobacter* spp., and LA-MRSA).” (Lines 1497-1501) On the issue of resistance zoonotic bacteria or identical bacterial DNA clones that can be selected by use of aminopenicillins, this RP presents data the results are equivocal where in some cases bacterial clones can be shown between animal and human isolates and in other cases no clear evidence indicates spread of identical bacterial clones from animals to	

Stakeholder no.	General comments	Outcome (if applicable)
	humans (this case is best stated for ESBL/AMPc *E. coli*). However, for zoonotic bacteria, this RP managed to state the following: - "Ampicillin resistance in *Salmonella* spp. ranges from nearly 4.1% (laying hens) to 44.7% (turkeys) in the EU with wide variation between the countries (from 0 to 87.5%). There is also variation in resistance between different salmonella serovars (Table 4; EFSA/ECDC, 2014, EFSA/ECDC, 2016). The occurrence of ESBL-/AmpC-producers in *Salmonella* spp. and indicator *E. coli* from poultry is uncommon (EFSA/ECDC, 2016). It should be noted, however, that indicator bacteria resistance figures are based on susceptibility testing of random bacterial colonies from non-selective media. In the case of specific ESBL/AmpC/carbapenemase monitoring, in which pre-enrichment and selective plating of specimens are used, the occurrence of ESBL/AmpC *E.coli* has been detected as very high in fattening turkeys (42%), broilers (47%), and in broiler meat (57%) with both wide variation in enzyme types as well as total occurrence between the countries (reference: EFSA/ECDC 2018)." (Lines 898-907) - "In an EFSA/ECDC report concerning the year 2014, seven EU/EFTA countries reported monitoring results for MRSA in food-producing animals or their environment and six countries reported results for MRSA in food of animal origin. In dairy cows, MRSA rates were 9.7% (Germany) and 16.9% (Netherlands), in pigs 0 – 60% (Iceland, Norway, Switzerland, Netherlands) and in turkeys 21.9% (Germany). MRSA was observed in meat from broilers (Switzerland), turkeys (Germany) and pigs (Spain) with a range of 3.2 - 42.5% positive batches, being the highest in turkey meat (EFSA/ECDC, 2016). The data is not comparable between the countries or even animal species within a country due to differences	

Stakeholder no.	General comments	Outcome (if applicable)
	in sampling methods and target populations." (Lines 920-927) - "Although aminopenicillins are not used for treating human salmonellosis, the data from 21 EU MSs and Norway in 2014 indicate that nearly one third of all *Salmonella* spp. isolates from human infections showed resistance to ampicillin (range 11.8-39.8%)." (Lines 1070-1072) - "A population study conducted in Italy in broiler chicken flocks, broiler meat, and humans demonstrated by whole genome sequencing and bioinformatics analysis that human cases of Salmonellosis by *S.* Infantis were caused by an emerging clone of ESBL (CXTM-1)-producing S. Infantis spreading in the broiler chicken industry since 2011, and that the ESBL gene was carried by a (IncP) conjugative mosaic megaplasmid (Franco et al., 2015)." (Lines 1437-1441) It is worth also concluding that current use of aminopenicillin (with inhibitors) combinations is low in the EU for food animals and that it should be encouraged that this consumption remains low in the EU, since this appears to be associated with positive effects of low prevalence of amoxi-clav resistance in food animals. This is further supported by statements in the RP. It is also worth concluding that aminopenicillin (with inhibitors) combinations formulations for mass medication purposes (e.g. premixes, drinking water products) should not be encouraged in the EU due to its potential devasting impact to further select and spread 3rd & 4th generation cephalosporin resistant bacteria. This is further highlighted by a statement in the RP, "However, the efficacy of amoxicillin-clavulanic acid compounds for treating systemic infections caused by *E. coli* or other Enterobacteriaceae in food-producing animals is questionable in relation to achievable drug concentrations *in vivo* with recommended dosage	

Stakeholder no.	General comments	Outcome (if applicable)
	schemes.” (Lines 1206-1208).	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
118-121	4	Comment: This sentence could be misinterpreted in that high genetic diversity does not relate to veterinary beta-lactam drug use. Also, the lack of genetic diversity can lead to misinterpretation. High genetic diversity demonstrates the complexity of the subject and bacteria harbouring ESBL/AmpCs. The RP should acknowledge that veterinary use of aminopenicillins might be involved in the mechanisms of maintenance and spread of ESBL/AmpC genes through horizontal transfer of mobile genetic elements (e.g. plasmids) in bacteria from food-producing animals and companion animals. It is well known that some ESBL-producing clones of "pure" zoonotic pathogens like some Salmonella serovars, e. g. *S. Infantis*, selected in food-producing animals are transferred to humans via the food chain and cause human disease. (See for instance Franco *et al*. 2015: Emergence of a Clonal Lineage of Multidrug-Resistant ESBL-Producing Salmonella Infantis Transmitted from Broilers and Broiler Meat to Humans in Italy between 2011 and 2014.)" http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0144802 Additionally, the semi-continuous use of oral aminopenicillins may also enhance the mutation rate of wild type beta-lactamase such as TEM-1 and SHV-1 that are widespread in *Enterobacteriaceae* from food-producing animals.	Agreed. We replaced the sentence by more recent findings. In the Netherlands, the most common plasmid types in humans also differ from those in livestock. This does not mean that there is no transmission between humans and animals, but that livestock is not the major source for humans. The section now reads: "Although identical clones, the same resistance genes and mobile genetic elements have been detected in many bacteria of animal and human origin, the effect of veterinary antimicrobial use on their presence or emergence in the human population is equivocal. A recent study comparing a large number of isolates failed to demonstrate a close epidemiological linkage of ESBL/AmpC genes and plasmid replicon types between livestock and people in the general population. In addition, a Dutch population-based modelling study estimated that most community-acquired ESBL/pAmpC-E. coli carriage was attributed to human-to-human transmission within or between households in the open community."

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Furthermore it may favour the spread of their mutants such as SHV-12 or TEM-52, which are true ESBL, and have been already reported in food-producing animals by almost all EU Members States. Since the usage of 3rd & 4th generation cephalosporins in poultry has never been authorised, then under current EU conditions, ESBL and AmpC-producing *E. coli* and Salmonella are likely to persist also by selection pressure with antibiotics other than 3rd & 4th generation cephalosporins. Nevertheless, the most prevalent genes encoding for ESBL (e. g. CTX-M-1 family, SHV-12, and AmpC (e. g. CMY2) in Enterobacteriaceae are located on transferable elements; this means that whether clones of the bacterial hosts in animals and humans are identical or not, is not relevant, since transferable genes from animal associated ESBLs can easily transfer to human bacterial clones. Proposed change (if any): The sentence requires further explanation to avoid misinterpretation.	
196-197	1	Comment: VetCAST acknowledges the great need for veterinary clinical breakpoints in general, also for aminopenicillins. We would like to elaborate the text by adding that such breakpoints should be animal species- and infection-specific, as this is crucial for veterinarians to obtain precise guidance on their choice of drugs. Proposed change (if any): "Susceptibility testing should be standardised and animal species- and infection-specific	Agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		veterinary clinical breakpoints should be established for aminopenicillins to enable proper interpretation of susceptibility tests."	
109-110	2	Comment: Please note a recent publication on this topic; M'Zali F. et al; 2018, AAVM 2018 Proposed change: Please take the publication into account and amend the sentence as necessary.	Not agreed. This publication is an abstract of a poster in the proceeding of a conference and not a peer reviewed full text publication.
172-74 1596-97	2	Comment: The Reflection Paper states repeatedly that "*Amoxicillin-clavulanate has wider spectrum and thus it is likely that it has higher chance to select multidrug resistant organisms compared to aminopenicillin alone.",* including in the final statement of the document Proposed change: Please add scientific references to substantiate the statement.	Agreed. We added a reference.
267-269	4	Comment: I believe that the exception to this list is *Clostridium difficile* which is typically resistant and aminopenicillins (e.g. amoxicillin) and aminopenicillin use are further considered high risk of being associated with 'antibiotic-associated diarrhea'. Proposed change (if any): Consider modifying the sentence.	Agreed. We amended the sentence. It now reads: Susceptible anaerobes include, among others, anaerobic Gram-positive cocci, most *Clostridium* spp. (except for *Clostridioides difficile*), *Fusobacterium* spp., *Prevotella* spp. and *Porphyromonas* spp.
327-329	4	Comment: You may consider modifying this sentence or separately describing the animal species. The reason why aminopenicillins cannot be administered orally differs in the animal species presented in this sentence. For example, in ruminants (foregut fermenter), the rumen microbes with	Agreed. We adapted the sentence: "Aminopenicillins cannot be administered orally to adult ruminants, horses or other animal species such as rabbits *and rodents due to degradation and severe disturbance of their*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		breakdown the aminopenicillin and none will be absorbed/available for pharmaceutical action. If high enough doses are given then the aminopenicillin could be associated with enterotoxemia. Whereas in horses and rabbits, oral aminopenicillins are associated with fatal colitis because of severe disturbances in gut microbiota due to fact these two animal species are hindgut fermenters. Proposed change (if any): Consider modifying the sentence.	*gut or rumen* microbiota.
433-434		Comment: The web-page cited lists only a subset of all beta-lactamases (only the TEMs, OXAs, and SHVs, the website page does not list the CTXs, etc.). To date, there are~2800 different beta-lactamases characterized. Due to the rapidly evolving science, we realize it is not straightforward to prevent this Reflection Paper from being outdated before it gets published. A disclaimer might therefore be warranted, or citation of a specific, recent 2018 review. Proposed change: Please update the reference. Suggested reference: Bush, K. 2018. Past and present perspectives on beta-lactamases. Antimicrob Agents Chemother. 62(10): e01076-18. https://aac.asm.org/content/aac/62/10/e01076-18.full.pdf	Agreed. The references have been updated.
538, Table 1	2	Comment: The title of the table and the 3rd column header have led to some confusion in interpretation by our members. The use of the term "antimicrobial targets" could be	Agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		interpreted to mean that these enzymes evolved or were selected in response to 'target' beta-lactam drugs. The footnote in the table provides more precise language to describe the content of column 3, i.e., that the beta-lactams listed in the third column of the table are hydrolyzed (at varying rates) by the group of enzymes listed in the first column. Indeed, we note that Bush and Jacoby who developed the Group classification and are cited in Table 1, do not use the term 'target' as it is currently applied in Table 1 of this draft Reflection Paper. Proposed change: Please amend the title. Examples of the most clinically relevant beta-lactamases, their beta-lactam ~~enzyme~~ substrates, ~~their target antimicrobials~~ and bacterial families or genera where present. The group classification is based on Bush and Jacoby's classification of beta-lactamases. Please amend the 3rd Column header: ~~Antimicrobial targets~~ Substrates	
593-595	4	Comment: Does EUCAST and/or CLSI have breakpoints for poultry pathogens (esp. E. coli)? I noticed that poultry are not mentioned in this sentence. If this is the case of no poultry breakpoints then it should be mentioned since poultry use of amoxicillin in the EU is high. APECs are sufficiently unique that adopting breakpoints from another species may not be representative. Proposed change (if any): Consider modifying the sentence to	No change needed. There are no breakpoints for poultry. We added this information: "*Even though use of amoxicillin in the EU is high in poultry no breakpoints are available as yet*."

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		mention poultry pathogens.	
Beta lactamases	4	Comment: It is worth pointing out that most beta-lactamases in Staphylococci are expressed extra-cellularly into the local environment providing a general protection in the local environment for a mixed infection. I believe that gram-negative retain beta-lactamases inter-cellularly, thus only providing individual benefits for the clone.	Not agreed. This seems of minor relevance to the RP.
607-632	2	Comment: It is very important that standard operating procedures (SOPs) and appropriate diagnostic measures are available in the veterinary field. This is essential in order firstly to avoid selecting existing resistance, potentially the development of new resistance strains by using empirically inappropriate molecules, secondly to reduce the global usage of antibiotics (antibiotics stewardship) and thirdly to prevent treatment failure. This strengthens the need for providing rapid and reliable diagnostic tools what could be reminded into the stated section. Proposed change: Please complete the observational paragraphs with the need for rapid and reliable diagnostic approaches.	Agreed. We added a sentence in the paragraph. It now reads: "It is very important that standard operating procedures (SOPs) and appropriate diagnostic measures are available in the veterinary field. This is essential in order to avoid selecting resistance, to reduce the global usage of antibiotics and to prevent treatment failure." In addition, this is mentioned in the recommendations: • Susceptibility testing should be standardised and animal- and infection- specific veterinary clinical breakpoints should be established for aminopenicillins to enable proper interpretation of susceptibility tests. • There is need for a harmonised European wide surveillance scheme to encompass target pathogens from food-producing and companion animals.
707-709	4	Comment: It is worth mentioning that the use of other extended spectrum penicillin classes (e.g. ticarcillin in the	Agreed. Sentence added: "Ticarcillin (not an aminopenicillin) for use in *Klebsiella* spp. infections is endorsed by CVMP on

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		uterus) is endorsed by the CVMP Essential list of substances for horses.	the essential list of substances for horses." (Regulation (EU) No 122/2013)
886-888	4	Comment: While this is true it is worth mentioning that the treatment of choice for animal streptococci is narrow-spectrum penicillins (e.g. benzylpenicillins), NOT aminopenicillins.	Agreed, but this is also true in humans. A sentence was added.
1144-1151	2	Comment: The study reported by Agerso Y. et al, 2014, has its limitation as there was no control group (with no usage of antibiotics). Caution should be exercised in attributing an increase of resistance to the use of aminopenicillins as stated by the authors "Use of aminopenicillins may influence the persistence of ESC-producing E. coli in the broiler production, but other factors should be investigated." Proposed change: Please amend the text to reflect the limitations of this study.	Not agreed. This study investigated the occurrence of extended spectrum cephalosporinase (ESC)–producing *Escherichia coli* in a broiler production with no cephalosporin use and a low use of antimicrobials in general.
1160-1163	4	Comment: I have concerns about this sentence. Firstly, Campylobacter are intrinsically resistant to 3rd generation cephalosporins. Selective agar media with 3rd generation cephalosporins are sometimes used by microbiology laboratories to isolate Campylobacter. Also, a scientific reference is given in support of the statement by Elviss et al., 2009. Upon examining this publication, it was noted in the summary the following: "In summary, data from this study indicate that amoxicillin treatment does not eradicate ampicillin/amoxicillin-resistant commensal Campylobacter from poultry and can select for ampicillin-resistant strains from a mixed population. This selection of ampicillin resistance may contribute to the predominance	Agreed. We amended the sentence. It now reads: "Capability to select resistance may not only depend on the substance, but also on the bacterial species in question since aminopenicillins have not proven to select for aminopenicillin resistance or resistance to critically important antimicrobials in *Campylobacter* spp., except if aminopenicillin-resistant Campylobacter are already present on the farm (Elviss et al., 2009; Juntunen et al., 2012).

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		of ampicillin-resistant Campylobacter in the food chain and in human infections." Thus, the publication by Elviss et al., 2009 states that amoxicillin treatment CAN select for ampicillin-resistance Campylobacter, which is opposite to what is stated in this RP. Proposed change (if any): Consider changing the sentence to reflect that amoxicillin treatment can select for ampicillin-resistant Campylobacter in poultry.	
1166-1167	2	Proposed change: Please amend amoxicillin and clavulanic acid throughout the document to (amoxicillin+clavulanic acid).	Not agreed.
1280-1282	2	Comment: Please also consider (Payet *et al;* 2018).	Not agreed. Reference is a proceeding of a conference and does not provide exhaustive information.
1293-1297	4	Comment: While I agree with the statements, it does not reflect the true risk/s to human exposed food of animal origin that is often contaminated by LA-MRSA. The consumption of this food may not substantially increase the risk of MRSA colonisation, but the handling of this food in the preparation process does increase risk of colonisation. This occurs despite standard food hygiene practices due environmental resilience of MRSA. Alcohol hand rubs are not standard food hygiene practices in households and thus there is a lack of risk mitigation measures from food of animal origin that is often contaminated with LA-MRSA. Proposed change (if any): Consider modifying the sentence to fully state the risks of exposures to foods of animal origin contaminated with LA-MRSA	Partly agreed. Some studies find this association, while other studies do not. We added the following sentence: "Handling meat has been associated with transmission in some studies (Boost et al.).while other studies did not find indications for transmission (Cuny et al. 2019)."

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
1485-1489	2	Comment: In our understanding of the sentence there is a grammatical error which leads to the whole list of antibiotics being taken as major selection forces. Proposed change: ~~Although~~ ~~t~~The major selection force for extended spectrum cephalosporin resistance is considered to be the use of cephalosporins and fluoroquinolones~~,~~; but aminopenicillins, especially inhibitor combinations, may co-select such resistance as can several other antimicrobials if	We amended the sentence.
	2	References: Agerso Y., Jensen JD., Hasman H., Pedersen K. Spread of extended spectrum cephalosporinase-producing *Escherichia coli* clones and plasmids from parent animals to broilers meat in a production without use of cephalosporins. Foodborne Pathogens and Disease. 2014, Vol 11(9): 740-746. Bush, K. 2018. Past and present perspectives on beta-lactamases. Antimicrob Agents Chemother. 62(10): e01076-18. https://aac.asm.org/content/aac/62/10/e01076-18.full.pdf M'Zali F., Hernoult M., Payet A., Prevel R., Arpin C., Dubois V., Kann M., Quentin-Noury C. Do all beta-lactams select for resistance equally? Frontiers in Microbiology. 2018: AAVM 2018) Payet A., ZabAla A., Bakour S., M'Zali F. Epidemiology of	No change needed. We prefer full text peer-reviewed publications.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Gram-negative organisms recovered from the littoral of South West of France. Proceeding of the International Conference Antimicrobial Agents in veterinary Medicine (AAVM); 16th - 19th October 2018, Rome- Italy published: 16 October 2018.	
1508-1510	4	Comment: The statement is rather unspecific and unnecessarily confusing to the issues presented. It is already known from ESVAC that the majority of aminopenicillin use is via oral administration, especially to food animals. Thus, it is unclear as to why the statement cannot be worded more specifically to the major route of administration in Europe. Also, it is unclear as to what animal densities encourage resistance selection and dissemination, under EU conditions. Proposed change (if any): Unless the statement can be stated more specifically (precisely) to the risk profiling of aminopenicillins, then it should be deleted.	Not agreed. Although oral use is the major route, other routes exist. High animal density favours spread of resistance between animals and between farms.
1529-1532	4	Comment: An example is given of a low risk estimate in relation to ampicillin-resistant *E. faecium*. However, it would be useful to also give an example of a high risk estimate, especially if it relates to aminopenicillins.	We were unable to find a reference for this specific example.
CTX-M	4	Comment: Under the issues of transmissible resistance from animal to human bacteria, then it is unclear as to why the previous story of CTX-M is not mentioned. Prior to 2010, there was a well-known global distribution of CTX-M15 in human clinical isolates. CTX-M1 was later identified in animals, but not humans and at one point CTX-M1 was considered animal-associated, whereas CTX-M15 was human-	Partly agreed. We added a few sentences on recent Dutch studies that indicate that human-to-human transmission is the most important transmission route. "Another study failed to demonstrate a close epidemiological linkage of ESBL/AmpC genes and plasmid replicon types between livestock farms and people in the general population

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		related. Around 2010 a Dutch study (can't remember the reference off-hand) was the first to report CTX-M1 in human clinical isolates and now commonly associated with human clinical isolates. If identical bacterial DNA clones are not associated with ESBL E. coli CTX-M1 from animal and human isolates then it at least is a story highlighting transmissible resistance transfer from mobile genetic elements.	(Dorado-García et al., 2017). *A Dutch population-based modelling study estimated that approximately 60% of community-acquired ESBL/pAmpC-E. coli carriage was attributed to human-to-human transmission within or between households in the open community. Secondary transmission from high-risk groups accounted for 7% of this carriage, food for 19%, companion animals for approximately 8%, farm animals (non-occupational contact) for 3·6% and swimming in freshwater and wild birds (ie, environmental contact) for 2·6% (Mughini-Gras et al., 2019). In another Dutch study, vegetarians and pescatarians did not have a lower risk of ESBL-E. coli/Klebsiella pneumoniae carriage compared with non-vegetarians, indicating that eating meat is not an important risk factor for ESBL-E.coli/Klebsiella pneumoniae carriage (Meijs et al., 2020).*