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Ad hoc method for the assessment of animal diseases caused by bacteria resistant to antimicrobials

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Abstract

The European Commission requested EFSA assess antimicrobial-resistant bacteria responsible for animal transmissible diseases, with a view to listing such pathogens for European Union action. This Scientific Opinion addresses the ad hoc method developed: (i) to give a global state of play as regards resistant animal pathogens that cause transmissible animal diseases, (ii) to identify the most relevant bacteria in the EU and (iii) to summarise their actual or potential animal health impact, and to perform their assessment for being listed and categorised according to the criteria of Articles 7, 5, 9 and 8 within the Animal Health Law (AHL) framework. An extensive literature review is carried out to give the global state of play of selected resistant bacteria that constitute a threat to animal health (i). An expert judgement procedure, based on the outcome of the literature review, is applied to identify which among those bacteria subjected to the literature review are the 'most relevant' in the European Union (ii). Their animal health impact in the European Union and their assessment for being listed and categorised according to the AHL framework will follow the 'ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law' that EFSA has developed in the past (iii). The assessment of (i) and (ii) is addressed in distinct scientific opinions that are published separately by animal species of interest (dogs and cats, horses, pigs, poultry, cattle, small ruminants, rabbits and aquatic animal species). The assessment of (iii) is addressed in distinct scientific opinions and published separately by the animal pathogen.

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1. Introduction

The Regulation (EU) 2016/429 on transmissible animal diseases ('Animal Health Law'),[1] from this point forward referred to as AHL, requires the Commission to list any disease after a completed assessment of that disease in accordance with the set of criteria provided for in the AHL. In the past years EFSA was asked several times to provide, and subsequently delivered, scientific opinions on the assessment of many diseases in animals, on the listing and categorisation of those diseases in accordance with the AHL. Parallel to this, the Commission adopted a European One Health Action Plan against Antimicrobial Resistance (AMR) in 2017, in which it committed to 'identify and assess under the AHL and with the support of EFSA, resistant bacteria that cause transmissible diseases and, if necessary, develop harmonised rules for their surveillance'.

1.1. Background as provided by the requestor

Provisions for the monitoring of antimicrobial resistance (AMR) in zoonotic and indicator bacteria are laid down in Directive 2003/99/EC[2]. Commission Implementing Decision 2013/652/EC[3], implementing that Directive, lays down detailed and harmonised rules for the monitoring and reporting of AMR in those bacteria. It is currently being revised, with an adoption foreseen for summer 2020. This legislative framework has been based on, and associated with, several past EFSA scientific outputs. Recently, EFSA published new Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food- producing animals and food.[4] There are no harmonised EU rules for the surveillance of AMR in bacteria, which cause transmissible animal diseases in animals, but not in humans. Indeed, before the AHL entered into force, the lack of suitable EU legal basis did not allow the establishment of specific rules for such EU-wide surveillance. Former AMR-related mandates to EFSA focused on AMR in food-borne bacteria in relation to their public health importance, although an EFSA-EMA joint scientific opinion also reviewed circumstances and diseases in which antimicrobials are most intensively used in food-producing animals and related examples of development of AMR.[5] In addition, EMA has provided advice on the impact on public health and animal health following the use of antimicrobials in animals.[6] This advice is being updated and should be finalised by the end of 2019/beginning of 2020.

There are relevant international standards, such as the Codes of the World Organisation for Animal Health (OIE), which provide guidance on the conduction of AMR monitoring and surveillance programmes in food-producing animals and list a number of bacterial pathogens as potentially to be included in a monitoring programme in food-producing animals. However, the relevant Chapter 6.8 of the Terrestrial Code[7] (Article 6.8.5. point 1, Table 2) does not provide an exhaustive list and in particular that point 1 and Table 2 contains only a few bacterial pathogens of animals (the focus of the current mandate) as examples. The corresponding Chapter 6.4. of the Aquatic Code[8] (Article 6.4.5.) lists only zoonotic bacteria as a minimum to be included in a monitoring programme.

In recent years, EFSA has been closely associated with the Commission services task aimed at the revision of the list of transmissible animal diseases in Annex II to the AHL, which will be applicable from April 2021. That included their thorough assessment against the specific criteria in the AHL and EFSA developed a methodology to contribute to that assessment.

Against this background, the Commission wishes to further explore this area, understand its state of play, and to the extent possible, explore emerging issues as regards AMR in bacteria responsible for transmissible animal diseases, with a view of listing such pathogens for EU action. The focus of this exercise is on resistant bacterial animal pathogens causing transmissible diseases excluding those already covered by Directive 2003/99/EC. The collection of such resistance data will provide a more

[1] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0429&rid=8
[2] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC, OJ L 325, 12.12.2003, p. 31.
[3] Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (notified under document C(2013) 7145) Text with EEA relevance, OJ L 303, 14.11.2013, p. 26.
[4] EFSA Journal 2019;17(6):570, https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5709
[5] E.g. EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA), https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4666
[6] https://www.ema.europa.eu/en/documents/other/answer-first-request-european-commission-scientificadvice-impact-public-health-animal-health-use_en.pdf
[7] www.oie.int/index.php?id=169&L=0&htmfile=chapitre_antibio_harmonisation.htm
[8] http://www.oie.int/index.php?id=171&L=0=htmfile=chapitre_antibio_development_harmonisation.htm

complete picture of antimicrobial resistance in animals. Together with the collection of data on antimicrobial sales and use per animal species under the new Regulation (EU) 2019/6 on veterinary medicinal products, it will strengthen integrated analysis and help better target policy measures promoting a prudent use of antimicrobials.

The Commission therefore needs first a comprehensive and global horizon screening, compilation and scrutiny of available data on existing cases and experienced problems related AMR in bacteria causing diseases in animals. Identification of lack of data, gaps and uncertainties is also important for this part of the exercise. This may include knowledge on the characterisation of AMR mechanisms and determinants responsible for the resistance in those bacteria.

Then the Commission needs advice specifically on the European dimension of the above global state of play. This advice should identify bacteria, which could be considered in the EU for particular attention by public authorities, animal keepers or other stakeholders alike, given their relevance. These above may include both bacteria already included in the list provided by Annex II to the AHL and any other animal bacteria.

Finally, the Commission needs scientific advice to decide which bacteria identified through the above process, if any, may qualify to be listed in the AHL for EU regulatory measures (e.g. notification, surveillance). This advice should be within the same framework of the already known listing and categorisation according to the AHL, in the same way it was delivered based on previous respective mandates. The criteria provided for in Articles 5, 7 and 8 and Annex IV of the AHL, shall be used as the basis for this analytical assessment.

1.2. Terms of Reference as provided by the requestor

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to provide a scientific opinion on the following:

1. Global state of play as regards resistant bacterial animal pathogens that cause transmissible animal diseases

To provide a state of play as regards AMR-related aspects in bacteria that cause transmissible animal diseases in terrestrial, aquatic or other animals.[9]

In particular, EFSA should perform a literature review as follows:

- Review AMR-related aspects of any bacteria responsible for transmissible animal diseases for which AMR concerns are described both at EU level and globally. This should include peer reviewed scientific literature but not necessarily limited to that, it may also assess information and data from other sources such as public/governmental sources, from livestock keepers or their organisations, industry, etc.
- Target animal species should include terrestrial and aquatic food-producing animals subject to farming in the EU, but also include companion animals, as there is a lack of data on AMR in those species.
- The scope of the review should be limited to antimicrobial resistant bacteria that constitute a threat to animal health, excluding bacteria covered by Directive 2003/99/EC.
- Based on the data available, the review should include a description of the occurrence and prevalence of AMR in such bacteria, the most relevant antibiotics against which resistance has developed and may also include other aspects such as the characterisation of the molecular mechanisms responsible for such resistance. Identify the most significant resistant bacteria causing disease in animals worldwide.
- Uncertainties and data gaps should be also identified and described.

2. Based on the global state of play established under point 1.

Summarise the situation in the EU in terms of the actual or potential impact on animal health of the most relevant bacteria in the EU, indicating those for which sufficient data exist and those for which data are not sufficient. EU relevance is to be understood on the basis of practical considerations, such as actual presence in the EU or presence elsewhere but in animal species, age groups or production systems which are widely used in the EU, or similar elements.

[9] Animal categories as defined in points (2), (3) and (4) of Article 4 of Regulation (EU) 2016/429 ("Animal Health Law").

3. Listing and categorisation of bacteria identified under point 2 in the framework of the Animal Health Law, where the available data seem to allow so:

3.1. Assess, following the criteria laid down in Article 7 of the AHL, their eligibility of being listed for Union intervention as laid down in Article 5(3)(a) and (b)(ii) of the AHL;

3.2. If found eligible to be listed for Union intervention, provide:

1) an assessment of their compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;
2) a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.

1.3. Interpretation of the Terms of Reference

This section includes the interpretation of Terms of Reference (ToR) 1, ToR 2 and ToR 3 of the mandate. Eight scientific opinions, one per host species or group of species (selected as explained below), covering the objectives of ToR 1 and the first part of ToR 2 (identification of most relevant bacteria in the EU) will be published following the methodology described in this Scientific Opinion. Should approaches or methodologies deviate in certain host-specific assessments, deviations will be described in detail in the corresponding scientific opinions.

To address the second part of ToR 2 (summarising actual or potential impact on animal health of the most relevant bacteria in the EU) and ToR 3 of this mandate, a single opinion covering each of the bacteria identified as relevant in the EU context in ToR 2 for all host species or group of species will be published. The interpretation and methodology specific to this ToR 3 is as in Section 1.2 of the Scientific Opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Articles 9 and 8 within the AHL framework (EFSA AHAW Panel, 2017).

1.3.1. ToR 1: Global state of play as regards resistant bacterial animal pathogens that cause transmissible animal diseases

In this first ToR, EFSA is requested to provide the state of play on AMR-related aspects in bacteria that cause transmissible animal diseases in terrestrial, aquatic or other animals according to the animal categories provided in Article 4 of the AHL. The scope of the mandate was limited to the following species in agreement with the European Commission:

- Cats and dogs
- Horses
- Swine
- Poultry (including chicken, turkey, duck, goose, game birds and ratites)
- Cattle
- Small ruminants (sheep and goat)
- Rabbits
- Aquatic animals (tilapia, carp, rainbow trout, bream).

In terms of the bacteria causing transmissible diseases in these animal species, EFSA was requested to exclude from this task zoonotic agents covered by Directive 2003/99/EC. Therefore, the following species were not included in the global state of play: *Borrelia* spp., *Brucella melitensis*, *B. abortus*, *B. canis* and *B. suis*, *Campylobacter jejuni* and *Campylobacter coli*, *Chlamydia psittaci*, *Clostridium botulinum*, *Leptospira* spp., *Listeria* spp., *Mycobacterium tuberculosis* complex members, *Salmonella enterica*, Verotoxigenic *Escherichia coli*, *Vibrio* spp. and *Yersinia* spp.

Furthermore, to define what was considered 'a bacterium responsible for transmissible animal diseases for which AMR concerns are described at EU level or globally', the EFSA working group (WG) agreed that:

only bacteria responsible for diseases that would trigger antimicrobial treatment, and for which evidence on the existence of clinical or microbiological AMR was available, should be included in the review.

Identification of such bacterial species was carried out through expert opinion (see Section 2.1.1). Similarly, the list of antimicrobials used to treat the infection caused by the evaluated bacterial pathogens was based on expert opinion and existing national antimicrobial guidelines, with a focus on

those published by European Member States (see Section 2.1.3). Finally, to retrieve the information that would allow to provide the global state of play, an extensive literature review that was then subjected to the assessment of the WG was performed.

1.3.2. ToR 2: Identification of the most relevant bacteria in the EU (among those included in ToR 1)

In the second ToR, EFSA is requested to summarise the actual or potential impact on animal health in the EU of the most relevant bacteria among those identified in ToR 1, defining relevance on the basis of practical considerations such as its actual presence in the EU, or elsewhere but in host categories/production systems widely represented in the EU.

The practical considerations used by the WG were the frequency of the diseases caused by the evaluated pathogens along with the frequency and geographic distribution of the AMR phenotypes with a focus on antimicrobials used for their treatment. The actual or potential impact of the antimicrobial resistant phenotypes of the bacteria (ARB) considered as 'most relevant' will be described and summarised according to the criteria already laid down in the EFSA opinion '*Ad hoc* method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law' (EFSA AHAW Panel, 2017) in which the impact on animal health has been defined by morbidity and mortality rates of the disease in the animal population and the severity of clinical signs at case level and related level and duration of impairment (table 3 in ref).

1.3.3. ToR 3: Listing and categorisation of bacteria identified under point 2 in the framework of the Animal Health Law

The most relevant resistant bacteria identified in ToR 2 will then be further assessed within the AHL framework. The AHL foresees the assessment of a disease/pathogen, based on the criteria listed in Articles 5 (eligibility for listing), Article 9 (categorisation into control classes) and Article 8 (listing species) performed on the basis of the information collected according to Article 7 (disease profile and impacts). EFSA has already delivered several scientific opinions on the assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law and developed an ad hoc methodology to guide the assessment (EFSA AHAW Panel, 2017). This ad hoc method will be applied to assess the eligibility of the most relevant bacteria identified in ToR 2 for being listed for European Union intervention (Article 5), their compliance with the criteria in Annex IV of the AHL for categorisation (Article 9) and assess the host species to be considered candidates for listing (Article 8).

2. Data and methodologies

EFSA clarified with the requestor the terms of reference received; the outcome of this step is reported in Section 1.2. Following clarification, the WG discussed the ToRs received from the requestor to have a clear understanding of the outcome, the approach and the data needs by ToR. The methodology laid down to answer each ToR is described in Sections 2.1, 2.2 and 2.3.

2.1. ToR 1: Global state of play as regards resistant bacterial animal pathogens that cause transmissible animal diseases

The process to address ToR 1 was based on an extensive literature review (ELR) to identify AMR-related aspects of relevant animal pathogens affecting terrestrial and aquatic food-producing and companion animals in the EU as requested. To define the scope of the literature review, the following steps were taken.

2.1.1. Definition of AMR concern

AMR concern for a given bacterial species in a given host species was defined as the existence of either evidence of:

- Reduced *in vitro* susceptibility of a bacterium to antimicrobials of categories B, C and D as laid down by the Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the European Medicines Agency (EMA) against which the bacterium is not naturally resistant;
- Reduced efficacy of antimicrobial treatment with antimicrobials of categories B, C and D of the AMEG list against the disease caused by this bacterium in a host species of interest.

Because the scope of this mandate only considers animal pathogens, it was the opinion of the WG that only resistance to antimicrobials authorised for use in livestock and companion animals in the EU were to be considered. Therefore, only antimicrobials included in the AMEG categories B ('restrict', use restricted for treatment of clinical conditions in the absence of alternative clinically effective antibiotics in lower categories and whenever possible based on results of antimicrobial susceptibility testing), C ('caution', use when there is no available options in category D that would be clinically effective) and D ('prudence', lowest risk to public health associated with their use in veterinary medicine, avoid unnecessary use/unnecessarily long treatment periods) were considered (EMA, 2020). The antimicrobials classes in these categories are listed in Table A.2 in Annex A. Resistance to category A antimicrobials ('avoid', antimicrobial classes not authorised in veterinary medicine) were not in the scope of the review.

Furthermore, it was agreed that focus should be made on antimicrobials that are important in clinical practice for the treatment of the infections caused by each bacterial pathogen. Existing treatment guidelines developed in European countries were used to advise the selection of these 'important antimicrobials for clinical practice'.

2.1.2. Bacterial species to be included in the ELR

Given that the scope of the review should be limited to antimicrobial resistant bacteria that constitute a threat to animal health, the WG experts drafted a list of bacterial species previously described as causative agents of infectious diseases in each of the host species of interest based on an informal review of the literature (without considering their clinical relevance). This process led to the identification of between 27 and 77 known pathogen species for each host species or group of host species (whole list of bacteria is reported in Appendix A).

Then, the known bacterial species that met one or more of the following conditions were excluded:

- The bacterium rarely causes diseases or production losses globally (i.e. where the bacterium is present in the world);
- The bacterium never or rarely leads to an antimicrobial therapy;
- The bacterium never or rarely causes antimicrobial treatment failure due to AMR.

Wording of these conditions was chosen to ensure that bacterial pathogens that would be relevant for the assessment were not excluded (i.e. maximised sensitivity of the approach at the possible cost of including non-relevant bacteria at a first stage). The screening was performed first by the WG members and then reviewed by a group of external experts in one or more of the host species of interest that were contacted by EFSA (experts are reported in the Acknowledgement section). External experts received the full list of bacteria for each host species and the preliminary assessment performed by the WG, and were asked to provide their opinion on whether each bacterium complied with each of the exclusion conditions stated above (possible answers: Yes/No/Unknown). The understanding of the terms 'never' or 'rarely' was left to the appreciation of the experts.

Overall, 15 experts responded to the survey, although they did not always provide answers for all host species (between four and eight external experts provided feedback for each host). Their answers (along with those from WG members) were then combined to obtain an overall judgement for each condition (Yes/No/Unknown).

Given that the objective of this process was to exclude only the bacterial species that were clearly outside of the scope of the review, only those bacteria in which a majority (> 50%) of the experts considered that it did not fulfil any of the exclusion criteria were deemed eligible. Obligate intracellular pathogens (e.g. *Anaplasma marginale*, *Coxiella burnetii*) were in principle excluded from the scope of the review due to the need to use cell models, which are time-consuming and labour intensive, to determine their susceptibility *in vitro* and therefore the expected reduced evidence available to perform a meaningful assessment of their AMR. The remaining list of pathogens was subjected to a second review by the WG to exclude potentially non-relevant pathogens still included in the list by considering the clinical relevance of the infection caused by each bacterium (i.e. removing bacterial pathogens with little clinical importance based on expert opinion), and to include other pathogens initially excluded by the external experts but deemed relevant by the WG.

The total number of bacteria that was finally included in the literature review ranged between 5 and 16 per host species (or group of host species) considered with the exception of aquatic species (in this case only those bacteria for which international harmonised cut-off values for resistance exist, were included) (CLSI, 2020) (Table 1). The full list of pathogens screened is reported in Appendix A.

Table 1: List of bacterial pathogens included in the literature review for each host species or group of species. Animal-associated pathogenic bacteria are defined based on the origin of clinical isolates

Host	Bacterial species	
Cats and dogs (n = 13)	*Bordetella bronchiseptica*	*Klebsiella pneumoniae*
	Clostridium difficile	*Proteus mirabilis*
	Clostridium perfringens	*Pseudomonas aeruginosa*
	Enterobacter spp.	*Staphylococcus aureus*
	Enterococcus faecalis	*Staphylococcus pseudintermedius*
	Enterococcus faecium	*Staphylococcus schleiferi*
	Escherichia coli	
Horses (n = 12)	*Actinobacillus equuli*	*Pseudomonas aeruginosa*
	Dermatophilus congolensis	*Rhodococcus equi*
	Enterococcus spp.	*Staphylococcus aureus*
	Escherichia coli	*Streptococcus dysgalactiae*
	Klebsiella pneumoniae	*Streptococcus equi equi*
	Pasteurella spp.	*Streptococcus equi zooepidemicus*
Swine (n = 16)	*Actinobacillus pleuropneumoniae*	*Mycoplasma hyorhinis*
	Bordetella bronchiseptica	*Mycoplasma hyosynoviae*
	Brachyspira hyodysenteriae	*Pasteurella multocida*
	Brachyspira pilosicoli	*Staphylococcus aureus*
	Erysipelothrix rhusiopathiae	*Staphylococcus hyicus*
	Escherichia coli	*Streptococcus dysgalactiae*
	Glaesserella (*Haemophilus*) *parasuis*	*Streptococcus suis*
	Mycoplasma hyopneumoniae	*Trueperella pyogenes*
Poultry (n = 14)	*Avibacterium paragallinarum*	*Gallibacterium anatis*
	Bordetella avium	*Mycoplasma gallisepticum*
	Clostridium perfringens	*Mycoplasma synoviae*
	Enterococcus cecorum	*Ornithobacterium rhinotracheale*
	Enterococcus faecalis	*Pasteurella multocida*
	Erysipelothrix rhusiopathiae	*Riemerella anatipestifer*
	Escherichia coli	*Staphylococcus aureus*
Cattle (n = 12)	*Escherichia coli* (non-VTEC)	*Mycoplasma bovis*
	Fusobacterium necrophorum	*Pasteurella multocida*
	Histophilus somni	*Staphylococcus aureus*
	Klebsiella pneumoniae	*Streptococcus dysgalactiae*
	Mannheimia haemolytica	*Streptococcus uberis*
	Moraxella bovis	*Trueperella pyogenes*
Sheep and goats (n = 16)	*Bibersteinia trehalosi*	*Mycoplasma capricolum capricolum*
	Campylobacter fetus	*Mycoplasma mycoides capri*
	Dichelobacter nodosus	*Mycoplasma ovipneumoniae*
	Escherichia coli (non-VTEC)	*Pasteurella multocida*
	Fusobacterium necrophorum	*Pseudomonas aeruginosa*
	Mannheimia haemolytica	*Staphylococcus aureus*
	Moraxella ovis	*Streptococcus uberis*
	Mycoplasma agalactiae	*Trueperella pyogenes*
Rabbit (n = 5)	*Bordetella bronchiseptica*	*Pseudomonas aeruginosa*
	Escherichia coli	*Staphylococcus aureus*
	Pasteurella multocida	
Aquatic species (n = 4)	*Aeromonas hydrophila*	*Flavobacterium columnare*
	Aeromonas salmonicida	*Flavobacterium psychrophilum*

2.1.3. Clinically relevant antibiotics

The antimicrobials included in the ELR were chosen among categories B, C and D, as laid down by the AMEG of the EMA (EMA, 2020). For each animal species/bacterial pathogen combination, relevant antimicrobials for veterinary therapy were selected, according to existing treatment guidelines from European countries and expert opinions. Each time, selected antimicrobials included first-line treatment options, as well as second- or third-line alternatives, even when experts were not aware of any resistance being described for the selected antimicrobials.

2.1.4. Extensive literature review (ELR)

To answer ToR 1, an ELS was outsourced by EFSA.[10] The aim of this ELS was to answer the epidemiological question:

'For which of the bacteria listed, is there evidence of acquired clinical resistance and/or reduced susceptibility to antimicrobial classes included in categories B, C or D defined by the Antimicrobial Advice ad hoc Expert Group (AMEG) of the European Medicines Agency (EMA) and recommended for treatment of the infection caused by them?'

To perform the task, the question was split into three subquestions that were used to build the search strings:

1) Does resistance to ANTIMICROBIAL DRUG NAME occur in BACTERIAL SPECIES NAME isolated from ANIMAL SPECIES NAME?
2) What is the frequency of resistance to ANTIMICROBIAL DRUG NAME in BACTERIAL SPECIES NAME isolated from ANIMAL SPECIES NAME?
3) Does resistance to ANTIMICROBIAL DRUG NAME in BACTERIAL SPECIES NAME cause treatment failure in ANIMAL SPECIES NAME?

Each of the searches was conducted separately for each host species/group of species, considering only the corresponding bacterial species listed in Section 2.1.2 as candidates. The ELR was limited to articles published after 2010, as the objective was to provide updated information that would describe the current global state of play, and was run on two international article databases, Web of Science (https://apps.webofknowledge.com/) and PubMed including Medline (https://www.nlm.nih.gov/bsd/pmresources.html).

To obtain data with sufficient quality to be used in the assessment, the following exclusion criteria were applied, mostly related to the way in which data on AMR were provided/summarised in the retrieved articles:

1) Data are provided in a way that does not allow one to differentiate between antimicrobial drugs (e.g. a study reports simply the antimicrobial class 'fluoroquinolones'). One exception is if the study lists the antibiotic tested in M&M (e.g. 'enrofloxacin') but reports data at class level ('fluoroquinolone').
2) AMR data are reported together for different animal species.
3) Data are reported at the bacterial genus level. Exceptions exist for bacteria often reported at the genus level and when species within the genus have the same breakpoints (e.g. *Enterococcus*, *Klebsiella*, *Pseudomonas*, *Proteus*).
4) Study is conducted not using a standard for antimicrobial susceptibility testing (AST), or a standard is not reported (CLSI, EUCAST or National Standards accepted).
5) The study provides results from non-clinical isolates (i.e. from non-clinically affected animals), or it is not possible to differentiate between clinical and non-clinical isolates.
6) The same individual is deliberately sampled multiple times.
7) The percentage of resistant isolates is not reported (e.g. data shown in bar charts instead).
8) Less than a minimum number of isolates are included in the study. This number may vary depending on the bacterial species (e.g. it can be as high as 50 in studies for *Staphylococcus* spp. and *E. coli*, in which a large number of references are expected, and lower for others). In any case, all studies including less than 10 isolates are excluded.
9) AMR data from the same isolates are included in another selected study (data duplication or a review article).

[10] https://ted.europa.eu/udl?uri=TED:NOTICE:457654-2020:TEXT:EN:HTML

10) Full text not available from searches using the journal subscriptions of the University of Copenhagen.
11) Articles are published in a language other than English.
12) Minimum inhibitory concentration (MIC) data are reported without interpretation into susceptible/resistant.
13) Antimicrobials included in the study are not routinely used for treatment in the host species based on available treatment guidelines.
14) The criteria for selection of the isolates are unclear and the risk of data duplication is high (e.g. studies testing diagnostic methods, comparing AST methods, defining clinical breakpoints or investigating pharmacodynamics of specific antimicrobial drugs).
15) All isolates in a study originate from the same farm or premises.
16) AMR was assessed only genotypically.
17) Other exclusion criteria (to be specified when appropriate).

These exclusion criteria could be modified, however, when considered appropriate in running the literature searches of specific host species (e.g. criterion 2: articles reporting data from isolates retrieved from cats and dogs without differentiating the source were considered eligible; criterion 16: studies in which the presence of the *mecA* gene was used to infer prevalence of methicillin-resistant *Staphylococcus aureus*, *S. pseudintermedius* were considered eligible). These modifications of the standard methodology will be reported in the scientific opinions devoted to each host species/group of species.

The data extracted from the selected articles included the number of isolates tested, their origin (e.g. type of sample from which they were isolated) when available, the number/proportion of isolates that was resistant to each antimicrobial tested among those considered of interest for the pathogen/host combination, and the interpretation criteria used to define resistance (clinical breakpoints or epidemiological cut-off values (ECOFFs)). This was used to produce a report summarising the results found in the ELR and tables reporting the raw extracted data. In addition, national AMR surveillance reports from EU countries including AMR data from clinical pathogens retrieved from the animal species of interest for this mandate will be also screened for eligibility using the same set of exclusion criteria as above. The protocol of this ELR was developed by the University of Copenhagen under the contract OC/EFSA/ALPHA/2020/02 – LOT 1[10]; further details on the protocol can be found in Annex A.

2.2. ToR 2: summary of the situation in the EU in terms of actual or potential impact on animal health of the most relevant bacteria in the EU

2.2.1. Assessment of the most relevant bacteria in the EU

The outcome of the ELR was used to first identify, among the screened bacterial species, the most relevant ones for the EU based on:

- the frequency of AMR in the bacterium (considering also the clinical relevance of the pathogen, number of publications – and isolates included in those publications – reporting the presence of resistant phenotypes, and the geographic distribution of the resistant isolates);
- availability of therapeutic options (range of antimicrobials to treat the infection and importance – first/second/third line – of the antimicrobials against which the bacteria were resistant).

Each WG member made an individual judgement on whether each of the bacterial species assessed in ToR 1 were among the 'most relevant' ones for a given host or not, or it was 'unclear', based on the evidence retrieved in the ELR, along with the rationale supporting their judgement. Individual judgements were then discussed at a WG meeting. Bacteria unanimously judged as 'most relevant' were automatically included in the following phase of the assessment (actual or potential impact on animal health under ToR2, and listing and categorisation under ToR 3), while those in which there was disagreement were subjected to further discussion until a consensus agreement was reached as to whether it should be included among the 'most relevant' category or not. Pathogens excluded from the 'most relevant' ones were further categorised based on their importance when possible according to the information available based on the evidence gathered and on the expert opinions (e.g. a higher number of studies/isolates tested was considered suggestive of higher clinical relevance of the pathogen and of resistant phenotypes).

2.2.2. Summary of the actual or potential impact on animal health in the EU of the most relevant bacteria

The actual or potential impact of the antimicrobial resistant bacteria considered as 'most relevant' will be described and summarised according to the criteria already defined in the EFSA opinion 'Ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law' (EFSA AHAW Panel, 2017).

2.3. ToR 3: Listing and categorisation of bacteria identified under point 2 in the framework of the Animal Health Law

The objective of this ToR is to assess the eligibility of the most relevant bacteria identified in ToR 2 for being listed for European Union intervention (Article 5), their compliance with the criteria in Annex IV of the AHL for categorisation (Article 9) and assess the host species to be considered candidates for listing (Article 8). To carry this out, EFSA will follow the ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law (EFSA AHAW Panel, 2017) developed in 2017 and implemented to perform the assessment of several animal diseases within the AHL framework (EFSA, online).

2.4. Uncertainty analysis

Several possible sources of uncertainty associated with different steps in the proposed methodology were identified:

- First, the initial list of pathogens was drafted through a non-systematic literature review, and therefore, there is a risk of both missing relevant pathogens and including pathogens non-relevant for this assessment.
- Second, although this list was further refined based on expert opinion to keep only pathogens that were relevant for the assessment in the ELR (global state of play as regards resistant bacteria), experts providing feedback mostly originated from the EU, and expertise was more limited for certain hosts or groups of hosts (e.g. only four external experts provided feedback for a subset of the pathogens initially considered for rabbits and aquatic animals).
- Third, the global state of play described in the host-specific scientific opinions as well as the identification of the most relevant bacteria for each host or group of hosts was carried out based on a ELR with a limited scope (e.g. only studies published in the last 10 years, in English or including some isolates – 10–50 depending on the bacterial pathogens – were included). It is therefore possible that we missed relevant literature not captured by the selection criteria defined for the ELR.
- Fourth, only resistance to antimicrobials in AMEG categories B, C and D was initially considered when defining AMR concern, since these are the only categories that are authorised for use in livestock and companion animals in the EU. However, resistance to other antimicrobials (AMEG category A) may also potentially influence or be associated with resistance to the selected antimicrobials (due to e.g. co-selection phenomena). Furthermore, even though available antimicrobial usage guidelines will be used to select the antimicrobials against which resistance will be assessed in the ELR, it is possible that certain antimicrobials that may be used to treat an infection by a given pathogen worldwide may be left out from the assessment.
- Fifth, since evidence retrieved originates primarily from studies published in the scientific literature, there is a risk of publication bias affecting the results found in the ELR (e.g. in favour of studies reporting higher levels of resistance).
- Finally, even though there was consensus on the criteria to consider for the identification of the 'most relevant' ARBs, and the literature review provided useful evidence on resistance proportions, the selection of the 'most relevant' ARBs was also based on expert opinion, as the lack of evidence obtained through unbiased and systematic methodologies would not allow using common or even host-specific thresholds for frequency of resistance in a given pathogen, geographical spread, etc.

Uncertainties derived from the data and the methodology used in the assessment could impact the conclusions in two ways:

- Certain ARBs could be wrongly included among the 'most relevant' ones for a given host ('false positive').
- Certain ARBs could be wrongly excluded from the 'most relevant' category for a given host ('false negative').

The probability of occurrence of false-positive or false-negative ARBs in the assessment was quantified collectively by expert judgement at the WG meetings taking into account all identified uncertainties. The impact of the sources of uncertainty that were identified on the final results of the scientific assessment was evaluated collectively by the WG using approximate probabilities (e.g. Table 2). Experts first provided individual judgements that were then discussed, and a consensus judgement was obtained.

Table 2: Approximate probability scale used to quantify overall uncertainty (see Table 2 in EFSA Scientific Committee, 2017)

Probability term	Subjective probability range	Additional options	
Almost certain	99–100%	More likely than not: > 50%	Unable to give any probability: range is 0–100% Report as 'inconclusive', 'cannot conclude' or 'unknown'
Extremely likely	95–99%		
Very likely	90–95%		
Likely	66–90%		
About as likely as not	33–66%		
Unlikely	10–33%		
Very unlikely	5–10%		
Extremely unlikely	1–5%		
Almost impossible	0–1%		

3. Conclusions

An ad hoc method for the assessment of animal diseases caused by bacteria resistant to antimicrobials was developed by the EFSA WG. The assessment methodology includes: (i) the procedure to identify the antimicrobial-resistant bacteria that may constitute a threat to animal health and the respective clinically relevant antimicrobials, (ii) the definition of the protocol to carry on an ELR to give a global state of play of those resistant bacteria and (iii) the procedure to carry on an expert judgement to identify the most relevant resistant bacteria in the EU.

This ad hoc method allowed the WG to identify between 5 and 16 ARB that constitute a threat to animal health in cats and dogs, horses, swine, poultry, cattle, small ruminants and rabbits, plus four ARB for aquatic animal species (the identified bacteria are reported in Table 1). A global state of play of those bacteria and a further assessment to identify those that might be considered as most relevant in the EU will be performed according to the method here presented. The assessment results will be published in a separated opinion, one for each host species (or group of host species).

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Abbreviations

AHL	Animal Health Law
AMEG	Antimicrobial Advice Ad Hoc Expert Group
AMR	Antimicrobial resistance
ARB	Antimicrobial-resistant bacterium
AST	Antimicrobial susceptibility testing
ELR	Extensive literature review
EMA	European Medicines Agency
MIC	Minimum inhibitory concentration
ToR	Terms of Reference
UTN	Universal Trial Number
WG	Working group

Appendix A – List of bacteria of interest by animal species presented in alphabetical order, indicating whether they were selected for inclusion or not

Table A.1: List of bacteria by animal species of interest sorted by included (in green) and not included (in grey) pathogens and alphabetical order (initial list and bacteria selected for the literature search)

Cats and dogs

Bacterium	Included in assessment?
Bordetella bronchiseptica	Yes
Clostridium difficile	Yes
Clostridium perfringens	Yes
Enterobacter spp.	Yes
Enterococcus faecalis	Yes
Enterococcus faecium	Yes
Escherichia coli	Yes
Klebsiella pneumoniae	Yes
Proteus mirabilis	Yes
Pseudomonas aeruginosa	Yes
Staphylococcus aureus	Yes
Staphylococcus pseudintermedius	Yes
Staphylococcus schleiferi	Yes
Acinetobacter baumannii	No
Actinomyces spp.	No
Actinomycetes viscosus	No
Anaplasma phagocytophilum	No
Anaplasma platys	No
Bartonella henselae	No
Bartonella spp. (*vinsonii* ssp. *berkhoffii*, *koehlerae*, etc.)	No
Borrelia burgdorferi	No
Burkholderia cepacia	No
Burkholderia pseudomallei	No
Campylobacter spp. (*upsaliensis* and *helveticus*)	No
Candidatus Neoehrlichia mikurensis	No
Chlamydia felis	No
Corynebacterium auriscanis	No
Coxiella burnetii	No
Ehrlichia canis	No
Ehrlichia spp. (*ewingii*, *chaffeensis*, *muris*)	No
Francisella tularensis	No
Helicobacter spp. (*felis*, *heilmanii*, others)	No
Mycobacterium microti	No
Mycoplasma canis	No
Mycoplasma cynos	No
Mycoplasma haematoparvum	No
Mycoplasma haemocanis	No
Mycoplasma haemofelis	No
Mycoplasma haemominutum	No
Mycoplasma turicensis	No
Nocardia asteroides	No
Pasteurella multocida	No

Bacterium	Included in assessment?
Rickettsia spp. (*felis*, *conorii*, *rickettsii*)	No
Staphylococcus spp. (CoNS)	No

Horses

Bacterium	Included in assessment?
Actinobacillus equuli	Yes
Dermatophilus congolensis	Yes
Enterococcus spp.	Yes
Escherichia coli	Yes
Klebsiella pneumoniae	Yes
Pasteurella spp.	Yes
Pseudomonas aeruginosa	Yes
Rhodococcus equi	Yes
Staphylococcus aureus	Yes
Streptococcus dysgalactiae subsp dysgalactiae/equisimilis	Yes
Streptococcus equi ssp. *equi*	Yes
Streptococcus equi ssp. *zooepidemicus*	Yes
Actinobacillus lignieresii	No
Actinomyces spp.	No
Anaplasma phagocytophilum	No
Bacillus anthracis	No
Bacteroides spp.	No
Bartonella henselae	No
Bordetella bronchiseptica	No
Borrelia burgdorferi	No
Burkholderia mallei	No
Burkholderia pseudomallei	No
Clostridium botulinum	No
Clostridium difficile	No
Clostridium perfringens	No
Clostridium piliforme	No
Clostridium spp. (*septicum*, *chauvoei*, *novyi*, *ramosum*, *sporogenes*, *fallax*)	No
Clostridium tetani	No
Corynebacterium pseudotuberculosis	No
Erysipelothrix rhusiopathiae	No
Francisella tularensis	No
Fusobacterium spp. (*necrophorum*)	No
Lawsonia intracellularis	No
Neorickettsia risticii	No
nocardia asteroides	No
Staphylococcus (*intermedius*)/(*pseudintermedius*)/*delphini*	No
Staphylococcus epidermidis	No
Taylorella equigenitalis	No

Swine

Bacterium	Included in assessment?
Actinobacillus pleuropneumoniae	Yes
Arcanobacterium pyogenes (*Trueperella pyogenes*)	Yes
Bordetella bronchiseptica	Yes

Bacterium	Included in assessment?
Brachyspira hyodysenteriae	Yes
Brachyspira pilosicoli	Yes
Erysipelothrix rhusiopathiae	Yes
Escherichia coli	Yes
Haemophilus parasuis (*Glässerella parasuis*)	Yes
Mycoplasma hyopneumoniae	Yes
Mycoplasma hyorhinis	Yes
Mycoplasma hyosynoviae	Yes
Pasteurella multocida	Yes
Staphylococcus aureus	Yes
Staphylococcus hyicus	Yes
Streptococcus dysgalactiae	Yes
Streptococcus suis	Yes
Trueperella pyogenes	Yes
Actinobacillus suis	No
Actinobaculum suis	No
Bacillus anthracis	No
Burkholderia pseudomallei	No
Chlamydia pecorum	No
Chlamydia trachomatis	No
Clostridium chauvoei	No
Clostridium difficile (*Clostridioides difficile*)	No
Clostridium novyi	No
Clostridium perfringens	No
Clostridium septicum	No
Clostridium tetani	No
Lawsonia intracellularis	No
Mycobacterium avium ssp. *hominissuis*	No
Mycoplasma suis	No
Rhodococcus equi	No
Streptococcus agalactiae	No
Streptococcus porcinus	No
Treponema pedis	No

Poultry

Bacterium	Included in the assessment?
Avibacterium paragallinarum	Yes
Bordetella avium	Yes
Clostridium perfringens	Yes
Enterococcus cecorum	Yes
Enterococcus faecalis	Yes
Erysipelothrix rhusiopathiae	Yes
Escherichia coli	Yes
Gallibacterium anatis	Yes
Mycoplasma gallisepticum	Yes
Mycoplasma synoviae	Yes
Ornithobacterium rhinotracheale	Yes
Pasteurella multocida	Yes
Riemerella anatipestifer	Yes

Bacterium	Included in the assessment?
Staphylococcus aureus	Yes
Acinetobacter spp.	No
Aegyptianella pullorum	No
Aeromonas hydrophila	No
Arcanobacterium pyogenes	No
Bacillus cereus	No
Brachyspira alvinipulli	No
Brachyspira hyodysenteriae	No
Brachyspira intermedia	No
Brachyspira pilosicoli	No
Campylobacter hepaticus	No
Clostridium colinum	No
Clostridium septicum	No
Enterococcus durans	No
Enterococcus faecium	No
Enterococcus hirae	No
Hafnia alvei	No
Helicobacter pullorum	No
Klebsiella spp.	No
Moraxella spp.	No
Mycobacterium avium ssp. *avium*	No
Mycoplasma gallinarum	No
Mycoplasma imitans	No
Mycoplasma iowae	No
Mycoplasma meleagridis	No
Mycoplasma pullorum	No
Proteus spp.	No
Pseudomonas aeruginosa	No
Pseudomonas fluorescens	No
Pseudomonas stutzeri	No
Riemerella columbina	No
Shigella flexneri	No
Shigella sonnei	No
Streptococcus bovis	No
Streptococcus dysgalactiae	No
Streptococcus equi ssp. *zooepidemicus*	No
Streptococcus mutans	No
Streptococcus pleomorphus	No

Cattle

Bacterium	Included in assessment?
Escherichia coli (*non-VTEC*)	Yes
Fusobacterium necrophorum	Yes
Histophilus somni	Yes
Klebsiella pneumoniae	Yes
Mannheimia haemolytica	Yes
Moraxella bovis	Yes
Mycoplasma bovis	Yes
Pasteurella multocida	Yes

Bacterium	Included in assessment?
Staphylococcus aureus	Yes
Streptococcus dysgalactiae	Yes
Streptococcus uberis	Yes
Trueperella pyogenes	Yes
Acinetobacter calcoaceticus	No
Actinobacillus lignieresii	No
Actinomyces bovis	No
Alcaligenes faecalis	No
Anaplasma centrale	No
Anaplasma marginale	No
Anaplasma phagocytophilum	No
Bacillus anthracis	No
Bacillus cereus	No
Bartonella bovis	No
Bartonella henselae	No
Bibersteinia trehalosi	No
Borrelia burgdorferi	No
Burkholderia pseudomallei	No
Chlamydia abortus	No
Chlamydia pecorum	No
Chlamydia spp.	No
Chlamydia suis	No
Citrobacter spp.	No
Clostridium chauvoei	No
Clostridium bifermentans	No
Clostridium difficile	No
Clostridium haemolyticum	No
Clostridium Novyi	No
Clostridium perfringens	No
Clostridium septicum	No
Clostridium sordelli	No
Clostridium sporogenes	No
Clostridium tetanii	No
Corynebacterium renale	No
Coxiella burnetii	No
Enterobacter cloacae	No
Enterococcus faecalis	No
Enterococcus faecium	No
Erysipelothrix rhusiopathiae	No
Gallibacterium anatis	No
Klebsiella aerogenes	No
Klebsiella oxytoca	No
Klebsiella ozaenae	No
Mannheimia varigena	No
Mycobacterium avium ssp. *paratuberculosis*	No
Mycoplasma bovigenitalium	No
Mycoplasma bovirhinis	No
Mycoplasma californicum	No
Mycoplasma canadense	No
Mycoplasma dispar	No

Bacterium	Included in assessment?
Mycoplasma mycoides ssp. *Mycoides*	No
Nocardia asteroides	No
Proteus mirabilis	No
Pseudomonas aeruginosa	No
Raoultella ornithinolytica	No
Raoultella terrigena	No
Serratia marcescens	No
Staphylococcus chromogenes	No
Staphylococcus epidermidis	No
Staphylococcus equorum	No
Staphylococcus haemolyticus	No
Staphylococcus sciuri	No
Staphylococcus simulans	No
Staphylococcus xylosus	No
Streptococcus agalactiae	No
Streptococcus pneumoniae	No
Streptococcus pyogenes	No
Ureaplasma diversum	No

Small ruminants

Bacterium	Included in the assessment?
Bibersteinia trehalosi	Yes
Campylobacter fetus	Yes
Dichelobacter nodosus	Yes
Escherichia coli (*non-VTEC*)	Yes
Fusobacterium necrophorum	Yes
Mannheimia haemolytica	Yes
Moraxella ovis	Yes
Mycoplasma agalactiae	Yes
Mycoplasma capricolum ssp. *capricolum*	Yes
Mycoplasma mycoides ssp. *capri*	Yes
Mycoplasma ovipneumoniae	Yes
Pasteurella multocida	Yes
Pseudomonas aeruginosa	Yes
Staphylococcus aureus	Yes
Streptococcus uberis	Yes
Trueperella pyogenes	Yes
Anaplasma spp.	No
Bacillus anthracis	No
Brucella ovis	No
Burkholderia cepacia	No
Chlamydia abortus	No
Chlamydia pecorum	No
Clostridium chauvoei	No
Clostridium novyi	No
Clostridium perfringens	No
Clostridium septicum	No
Clostridium sordellii	No
Clostridium tetani	No

Bacterium	Included in the assessment?
Corynebacterium pseudotuberculosis	No
Corynebacterium renale	No
Coxiella burnetii	No
Dermatophilus congolensis	No
Ehrlichia ruminantium	No
Francisella tularensis	No
Klebsiella pneumoniae	No
Mycobacterium avium ssp. *paratuberculosis*	No
Mycoplasma arginini	No
Mycoplasma capricolum ssp. *capripneumoniae*	No
Mycoplasma conjunctivae	No
Prevotella melaninogenica	No
Proteus mirabilis	No
Pseudomonas pseudomallei	No
Serratia marcescens	No
Staphylococcus caprae	No
Staphylococcus chromogenes	No
Staphylococcus epidermidis	No
Staphylococcus simulans	No
Staphylococcus xylosus	No
Streptococcus agalactiae	No
Streptococcus suis	No

Rabbits

Bacterium	Included in the assessment?
Bordetella bronchiseptica	Yes
Escherichia coli	Yes
Pasteurella multocida	Yes
Pseudomonas aeruginosa	Yes
Staphylococcus aureus	Yes
Actinobacillus spp.	No
Actinomyces spp.	No
Bacillus sp.	No
Campylobacter spp.	No
Clostridium difficile	No
Clostridium perfringens	No
Clostridium piliforme	No
Clostridium spiroforme	No
Corynebacterium sp.	No
Filobacterium rodentium (CAR *Bacillus*)	No
Francisella tularensis	No
Fusobacterium necrophorum	No
Fusobacterium nucleatum	No
Lawsonia intracellularis	No
Micrococcus sp.	No
Moraxella sp.	No
Mycobacterium avium	No
Mycobacterium avium ssp. *paratuberculosis*	No
Neisseria sp.	No

Bacterium	Included in the assessment?
Stomatococcus sp.	No
Streptococcus sp. (*S. pyogenes*)	No
Treponema paraluiscuniculi	No

Aquatic animals: only bacterial species for which international harmonised cut-off values for resistance exist were included

Bacterium	Included in the assessment?
Aeromonas hydrophila	Yes
Aeromonas salmonicida	Yes
Flavobacterium columnare	Yes
Flavobacterium psychrophilum	Yes

Annex A – Protocol for the Extensive Literature Review

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A.1. Description of the task

The task is to carry out an Extensive Literature Review (ELR) to answer the epidemiological question: *For which of the bacteria listed in Table A.1, is there evidence of acquired clinical resistance and/or reduced susceptibility to antimicrobial classes included in Categories B, C or D defined by the Antimicrobial Advice ad hoc Expert Group (AMEG) of the European Medicines Agency (EMA)?* The ELR is expected to gather the relevant literature to answer this question with particular regard to occurrence, frequency and geographical distribution of AMR at the global level for each bacterial species listed in Table A.1. The team at the University of Copenhagen (UCPH) will carry out this task according to the time plan agreed with EFSA, and will deliver a separate ELR for each of the following animal species/groups:

- Dogs and cats (ELR 1)
- Horses (ELR 2)
- Pigs (ELR 3)
- Poultry (chicken and turkey, duck, goose, game bird, ratites (ELR 4)
- Cattle (ELR 5)
- Sheep and goats (ELR 6)
- Rabbit (ELR 7)
- Aquatic animals (ELR 8)

The task includes the following three subtasks:

Task 1 – Develop a tailored search strategy (including terms and strings as well inclusion/exclusion criteria) for answering the question mentioned above.
Task 2 – Carry out an ELR using the search strategy and following the protocol described in this document.
Task 3 – Extract and synthetise the data retrieved by each ELR.

A.2. Review questions

Clear review questions are indispensable to perform this work since AMR is a generic term that requires contextualisation (i.e. animal species, bacterial species/genus and antimicrobial drug name). Accordingly, we propose to split the question of Task 1 into three questions:

1) Does resistance to ANTIMICROBIAL DRUG NAME occur in BACTERIAL SPECIES NAME isolated from ANIMAL SPECIES NAME?
2) What is the frequency of resistance to ANTIMICROBIAL DRUG NAME in BACTERIAL SPECIES NAME isolated from ANIMAL SPECIES NAME?
3) Does resistance to ANTIMICROBIAL DRUG NAME in BACTERIAL SPECIES NAME cause treatment failure in ANIMAL SPECIES NAME?

A.3. Eligibility criteria

Inclusion criteria (before full-text review):

- Bacterial species/genus: only bacterial species and genera listed in Table A.1.
- Antimicrobial drug: only antimicrobial classes belonging to categories B, C or D in the AMEG classification.
- Publication year: only articles, reports, etc. published between 2010 and 2020.
- Language and accessibility: only publications in English available in full text from the UCPH server.
- Type of study: any *in vitro* studies providing phenotypic data on antimicrobial susceptibility testing or genotypic data on presence/absence of AMR genes.

Exclusion criteria (may be applied during abstract screening, but often not possible to follow before full-text review):

- Not possible to differentiate between antimicrobial drugs (e.g. a study reports antimicrobial classes 'fluoroquinolones'). One exception is if the study lists the antibiotic tested in M&M (e.g. 'enrofloxacin') but reports data at class level ('fluoroquinolone').
- AMR data are reported together for different animal species (exception: dog/cat and sheep/goat may be reported together).

- Data reported at the genus level. Exceptions exist for bacteria often reported at the genus level and where species within the genus have the same breakpoints (e.g. *Enterococcus*, *Klebsiella*, *Pseudomonas*, *Proteus*).
- Not using a standard for AST or standard not reported (CLSI, EUCAST or National Standards accepted).
- Not clinical isolates, or not able to differentiate between clinical and non-clinical isolates
- Same individual deliberately sampled multiple times.
- No % resistant reported (e.g. data shown in bar charts instead).
- Less than 10 isolates in a study. NB: in order to increase quality of extracted data, this lower may be increased for some commonly occurring pathogens, e.g. *Staphylococcus* spp and *E. coli*.
- AMR data from the same isolates are included in another selected study (data duplication or a review).
- Full text not available from searches using the journal subscriptions of the University of Copenhagen.
- Non-English.
- MIC data reported without interpretation into susceptible/resistant.
- Antibiotics reported are not routinely used for treatment in the host species based on available treatment guidelines (list of clinically relevant antibiotics is made and agreed upon with the EFSA WG before extraction of data).
- Studies where the criteria for selection of the isolates are unclear and the risk of data duplication is high (e.g. studies testing diagnostic methods, comparing AST methods, defining clinical breakpoints or investigating pharmacodynamics of specific antimicrobial drugs).
- All isolates in a study originate from the same farm/house.
- Studies where AMR was only assessed genotypically except those where *mecA* was used to infer MRSA/MRSP prevalence.
- Other (reasons to be added in Excel file).

Outcome measures:

- AMR occurrence (yes/not) and AMR frequency/prevalence, which is usually reported as a fraction or percentage (e.g. number of resistant isolates divided by the total number of isolates tested).

A.4. Information sources

Two international article databases, EMBASE and PubMed, will be searched using the search strategy described in Section A.5. To minimise the risk of data duplication, grey literature will be limited to reports from national or international AMR surveillance programmes.

A.5. Search strategy

Search strategies were developed to address the specific questions formulated in Section A.2 for each of the bacterial species listed in Table A.1 of this Appendix. All searches were performed in PubMed and Embase databases. For each database and animal species, we introduced terms that targeted (i) antimicrobial agents, (ii) resistance/susceptibility, (iii) animal host and (iv) bacterial pathogen. In PubMed, each term was included as a title/abstract term and as a subject heading (MeSH) term (if available). Review papers and publications prior to 2010 were excluded from the final search. In Embase, each term was included as a multi-purpose term (mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word) and as a subject heading term (if available). For each independent term searched as a subject heading, narrower terms were examined, and specific narrower terms may have been included if relevant. In lack of specific terms as subject headings, broader terms were also examined and included if relevant and if the search did not broaden to other pathogens not aimed for (e.g. we included the subject heading term 'Lawsonia bacteria' instead of 'Lawsonia intracellularis', which did not exist as a subject heading). Detailed search strings will be provided in the separate reports (**D1.2**) for each animal investigated.

Although the above search string does not contain terms related to treatment outcome, we believe that papers on that topic (Type B studies) will also appear from the string. After all, treatment outcome has to be linked to data on antimicrobial susceptibility.

A.6. Data management

The results of search engines will be downloaded and load them into the Rayyan software (https://libraryguides.mcgill.ca/rayyan/gettingstarted). Duplicate elimination and selection based on title/abstract will be performed in Rayyan. The final list of publications that will be checked in full text can be exported from Rayyan in RefMan, BibTex, EndNote or csv formats, which can be shared between reviewers.

A.7. Selection process

The initial selection process will be executed by a reviewer with expertise in AMR. In the first instance, abstracts and titles will be screened according to the inclusion criteria listed in Section A.3. The reviewer will evaluate each reference for relevance using the following sequence of questions:

1) Does the title or abstract refer to *in vitro* phenotypic or genotypic data on AMR/susceptibility in relevant bacterial species/genus?

Yes → Include for full-text assessment
No → Exclude

Following title/abstract screening, the selected studies will be included in the ELR when appropriate. The full text of the potentially relevant publications will be retrieved and assessed for compliance to the eligibility criteria listed in Section A.3. Only references passing this assessment step will be included in the data extraction.

Identification and deletion of duplicate data, i.e. data that have been included in multiple publications. Methods to help identify duplicated data:

1) Trial identification numbers (e.g. ClinicalTrials.gov Identifier (NCT number); ISRCTN; Universal Trial Number (UTN); other identifiers such as those from the sponsor.
2) Author names.
3) Location and setting.
4) Specific details of the interventions (e.g. dose, frequency).
5) Numbers of participants and baseline data.
6) Date and duration of the study which can also clarify whether different sample sizes are due to different periods of recruitment.

The selection process is a very critical step since relevant studies and useful data might be lost due to human errors or methodological inaccuracies. The quality assurance methods that we propose to minimise this risk are described in Section A.10.

A.8. Data extraction

The data extraction process will be performed by a dedicated 'data extractor'. Data will be transferred to an electronic database extraction form shared by data reviewers in Google Sheets. The number of free text fields will be limited as much as possible to simplify data analysis. This data extraction form, or an extraction thereof into a table format, will function as deliverable **D1.3**.

For each paper deemed to meet the criteria for inclusion, the information mentioned in Section A.10 will be extracted.

A.9. Data synthesis

Data extracted from the included studies for each animal species will be summarised in a summary report. This narrative synthesis will be used to highlight key differences between the studies in relation to study design, population, methodology, etc. as well as to assess their methodological quality. For each animal species, the studies will be grouped by production type, bacterial pathogen and outcome measure, since it does not make sense to compare AMR studies that differ in relation to these factors. The results will be tabulated in a summary table including results separated by relevant groups (e.g. age groups, disease type), and add comments concerning relevant methodological differences

between studies, or differences in the populations studied. The report will highlight knowledge gaps, and potential biases in the review process will be evaluated, i.e. if the methods used to perform the review and the assumptions made to analyse the data may have affected the results.

A.10. Quality assurance

The following measures will be taken to ensure quality and reduce bias or human errors during the critical steps of the review process.

Selection of studies

- Studies will be selected according to the inclusion and exclusion criteria described in Section A.3.
- The selection process will be described in the summary report including a description of the main reasons for exclusion of studies after full-text screening.

Data extraction

- A data extraction form implementing the key points in Section A.10 will be used to ensure consistency, and improve validity and reliability of the extraction process.
- The data extraction form will be piloted on a sample of included studies to ensure that all the relevant information is captured and that resources are not wasted on extracting data that are not required.
- To minimise data extraction errors and ensure a sufficient and harmonised level of detail, reviewers will regularly check the data extractors' input to the data extraction form. If disagreements occur between the reviewers and data extractors, they will be resolved using a consensus-based approach after discussing disagreements.

Assessment of study quality

- The reviewers will assess – individually and through discussion with relevant experts in the consortium – the risk of bias caused by inadequacies in study design, conduct or analysis that may lead to errors in the measurement or misinterpretation of antimicrobial susceptibility data (type A studies), or to over/underestimation of the treatment outcome (type B studies).
- It will be noted for each study whether the research question was stated clearly or not.
- It will be assessed if the population of a study was clearly specified and defined. In order to assess that somewhat objectively, studies will be divided into i) those with isolates deriving from a clearly defined population of animals in a clinic, hospital, farm or similar (e.g. kennel), and ii) those from a diagnostic laboratory with a non-defined population (i.e. without background information on patients).

Table A.2: AMEG categorisation of antimicrobials classes

Category B	3rd and 4th gen. cephalosporins cefoperazone cefovecin cefquinome ceftiofur colistin polymyxin B	Polymyxins cinoxacin danofloxacin difloxacin enrofloxacin flumequine ibafloxacin marbofloxacin norfloxacin Orbifloxacin oxolinic acid pradofloxacin	Quinolones: cinoxacin danofloxacin difloxacin enrofloxacin flumequine ibafloxacin marbofloxacin norfloxacin Orbifloxacin oxolinic acid pradofloxacin
Category C	Aminoglycosides (except spectinomycin) amikacin apramycin dihydrostreptomycin framycetin gentamicin kanamycin neomycin paromomycin streptomycin tobramycin	1st and 2nd gen. cephalosporins, and cephamycins cefacetrile cefadroxil cefalexin cefalonium cefalotin cefapirin cefazolin	Macrolides erythromycin gamithromycin oleandomycin spiramycin tildipirosin tilmicosin tulathromycin tylosin tylvalosin
	Aminopenicillins with beta-lactamase inhibitors amoxicillin + clav. acid ampicillin + sulbactam	Lincosamides clindamycin lincomycin pirlimycin	Rifamycins: rifaximin only rifaximin
	Amphenicols chloramphenicol florfenicol thiamphenicol	Pleuromutilins tiamulin valnemulin	
Category D	Aminopenicillins, without beta-lactamase inhibitors amoxicillin ampicillin metampicillin	Anti-staphylococcal penicillins (beta-lactamase-resistant penicillins) cloxacillin dicloxacillin nafcillin oxacillin	Sulfonamides, dihydrofolate reductase inhibitors and combinations formosulfathiazole phthalylsulfathiazole sulfacetamide sulfachlorpyridazine sulfaclozine sulfadiazine sulfadimethoxine sulfadimidine sulfadoxine sulfafurazole sulfaguanidine sulfalene sulfamerazine sulfamethizole sulfamethoxazole sulfamethoxypyridazine sulfamonomethoxine sulfanilamide sulfapyridine sulfaquinoxaline sulfathiazole trimethoprim
	Aminoglycosides: spectinomycin only spectinomycin	Natural, narrow-spectrum penicillins (beta lactamase-sensitive penicillins) benzathine phenoxymethylpenicillin benzylpenicillin penethamate hydriodide pheneticillin phenoxymethylpenicillin procaine benzylpenicillin	
	Cyclic polypeptides bacitracin		
	Nitroimidazoles metronidazole		
	Nitrofuran derivatives furaltadone furazolidone	Tetracyclines chlortetracycline doxycycline oxytetracycline tetracycline	
	Steroid antibacterials Fusidic acid		

A.11. Key points in planning data extraction

The following information will be extracted and typed into an Excel sheet for publications/reports selected for full-text evaluation:

- Study ID
- Initials of person screening full text
- Decision to include or not

- Specifically for excluded studies:
  - Reason(s) for exclusion

- Specifically for included studies:
  - Whether the research question was clearly stated or not
  - Study population category:
    - 1: Clinic/hospital/farm
    - 2: Diagnostic lab (i.e. unknown population)
  - If the study provided raw data (e.g. MIC data)
  - Method for detection/quantification of resistance
  - Method for bacterial species identification
  - Country
  - Animal species
  - Publication year
  - Bacterial species
  - How the intermediate category was reported/used
  - Organ
  - Antibiotic
  - Number of isolates
  - % resistant isolates
  - % intermediate isolates
  - % intermediate + resistant isolates
  - Reference for breakpoint used or epidemiological cut-off used
  - Various notes to explain data (when needed)

Other variables may be added ad hoc for specific animal species, e.g. 'production type' (e.g. dairy/ meat/egg) and 'production system' (e.g. conventional/organic) will be relevant for food-producing animals but not for companion animals.

The 'Year' of bacterial isolation will not be neither extracted nor reported from the studies included, as in most studies, the isolates had been collected over several years with no specification on the number of isolates per year.