Final version

13/03/2018

Advisory Committee on the Microbiological Safety of Food

Fixed-term task and finish group on antimicrobial resistance

Antimicrobial resistance in the food chain; research questions and potential approaches

Advises the Food Standards Agency on the Microbiological Safety of Food

Membership

ACMSF AMR Working Group Members	
Chair Prof David McDowell	ACMSF member, chair of the ACMSF AMR working group, Emeritus Professor of Food Studies University of Ulster
Dr Dan Tucker	ACMSF member, senior lecturer in Veterinary Public Health/pig medicine at the Department of Veterinary Medicine, University of Cambridge.
Prof Rick Holliman	Co-opted member (formerly Public Health England and ACMSF Member)
Prof John Coia	Co-opted member, Consultant Clinical Microbiologist with NHS Greater Glasgow and Clyde, Director of the Scottish Microbiology reference laboratories, Glasgow and formerly ACMSF member
Prof Stephen Forsythe	Co-opted member (Advisory Committee on Animal Feeding stuffs member)
Mr Chris Teale	Co-opted member. Veterinary medicine. (Animal and Plant Health Agency)
Prof John Threlfall	Co-opted member (ex-PHE). AMR expert has published extensively. Expertise includes, AMR, enteric microbiology, Microbial genetics, molecular epidemiology, microbial risk assessment.
Mr Stephen Wyllie	Veterinary representative on ACMSF (Animal and Plant Health Agency)
Additional Members/Observers on the Task and Finish Group	
Prof Dov Stekel	Co-opted member, School of Biosciences, University of Nottingham. Using mathematical and computer models at both molecular and population levels to study mechanisms for, and spread of, AMR.
Prof Roberto La Ragione	Co-opted member, School of Veterinary Medicine, University of Surrey Veterinary microbiology, AMR transmission, alternatives to antimicrobials.
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The terms of reference of the group are as follows:

To identify research questions and potential approaches which would (i) decrease uncertainty about any linkage between use of antimicrobials in food production, the incidence of antimicrobial resistance in pathogens and commensals in food production, and the growing AMR-related public health burden, and (ii) allow us to model the impacts of changes in use of antimicrobials in food production. Poultry, sheep, cattle and pigs will be covered in the scope.

Executive Summary

The trends seen in the development and dissemination of antimicrobial resistance (AMR) poses significant public health challenges in many aspects of human activities, including the human food chain.

In early 2017 following a request from the Food Standards Agency, the ACMSF established a fixed term (<1 year) Task and Finish Group comprised of the existing ACMSF AMR subgroup, supplemented with wider AMR expertise to identify research questions and potential approaches for future research and related activities relating to antimicrobial resistance and the food chain.

Bearing in mind the fixed term nature of the Task and Finish Group, and the diverse and interrelated impacts of the wider emergence and dissemination of AMR, the group's terms of reference focussed on antimicrobials and AMR in food production with particular reference to the activities and responsibility of the FSA, i.e.

i. To identify research questions and potential approaches which would decrease uncertainty about any linkage between use of antimicrobials in food production, the incidence of antimicrobial resistance in pathogens and commensals in food production, and the growing AMR-related public health burden, and;

ii. allow us to model the impacts of changes in use of antimicrobials in food production.

This group met 5 times during 2017. During a series of scoping discussions, the group worked closely with colleagues from FSA Risk Assessment and Analytics, in reviewing the FSA relevant aspects of an AMR systems map developed by DoH, PHE, DEFRA and VMD in 2014, and in developing a food chain focussed AMR systems map. This map guided the discussions and activities of the group, and identified eight main reservoirs with a potential AMR impact relevant to FSA, which were subsequently reviewed within our report. As part of this review process, the group also received presentations on antimicrobial usage and AMR from a number of UK food animal production sectors (poultry, pigs, dairy and beef cattle, sheep).

The eight main reservoirs of relevance to FSA research questions were identified as:

- Pasture & Crops
- Amendments
- Animal Feed
- Food Producing Animals
- Abattoir & Carcass Processing
- Food Processing
- Human Food
- Humans

In line with the terms of reference provided by FSA, our review of each of the above reservoirs identified key areas, and timely appropriate actions to be considered by FSA, in relation to the Agency's interests and responsibilities, i.e. "no action", "lead action", "encouragement/collaboration" or "watching brief".

The group also

- derived a number of general conclusions and overarching themes including the need for more data on AMR in relation to; co-ordinated (One Health) regular, targeted surveillance of UK produced/processed and imported foods; AMR transfer between commensals and pathogens; the potential impact of Brexit; alternatives to antimicrobials; the AMR selective effects of feed/food processing actions and environments.

- noted the value of co-ordinated use of more discriminatory systems (WGS /bioinformatics) in understanding the epidemiology and ecology or AMR in the human food chain, and the need to review current hygiene activities with particular reference to AMR. The group recognised the considerable industry emphasis on, (and progress in) antimicrobial stewardship, and highlighted the importance of similar progress in other important aspects of AMR control, such as biosecurity, animal and plant husbandry, use of alternative AMR agents, and prevention of infection/cross contamination during food production.

The group hopes that the structured reviews of the identified AMR reservoirs, and the overarching themes, presented in this report will provide a rational and effective framework for research planning and activities in understanding and controlling the AMR-related public health burden associated with the UK food chain.

Introduction

1. Antimicrobial resistance (AMR) is a significant public health issue with the potential to have impacts on a global scale. International organisations such as WHO, FAO, the World Organisation for Animal Health (OIE) and the European Commission have also recognised the potential threat posed by AMR and have published action plans seeking to address this problem. Very few new antimicrobials are becoming available in the foreseeable future so conserving the efficacy of our current antimicrobial drugs is crucial in treating infections. Addressing the risks of AMR is a priority for the UK Government and the devolved administrations, which are committed to an integrated approach at national and international levels, through actions set out in the UK Five Year Antimicrobial Resistance Strategy (DoHSC, 2013).

2. AMR can lead to therapeutic failure and increased morbidity and mortality among individuals with infections caused by drug-resistant pathogens. It has been estimated that worldwide, 700,000 people die every year from drug-resistant strains of common bacterial infections, although this number is probably an underestimation due to poor reporting and surveillance. Unless effective action is taken, the burden of deaths from AMR could balloon to 10 million lives each year by 2050 (O'Neill, 2016). Human exposure to drug-resistant bacteria can occur via many routes, including person-to-person transmission, direct contact with animals, and the environment as well as through the food chain. The complexity and interrelatedness of this issue is illustrated by Figure 1 below.

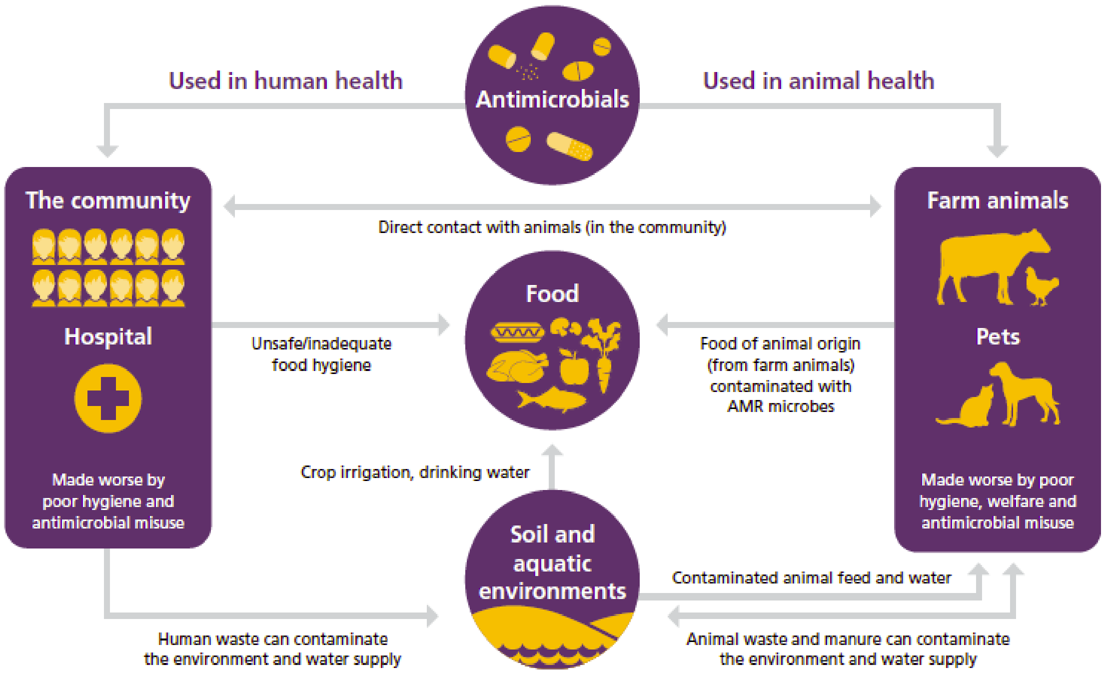

Figure 1: How antimicrobial resistance can spread through food[1]

[1] See https://www.food.gov.uk/news-updates/news/2017/16629/final-results-third-annual-retail-survey

3. There has been a longstanding interest in the contribution that the food chain makes to the problem of AMR bacteria in humans. ACMSF (ACMSF, 1999) noted some evidence that AMR foodborne pathogens such as *Salmonella* spp. and *Campylobacter* spp. contribute to human infections but the magnitude of these contributions and the impact of other AMR bacteria, including commensals, remain uncertain. Some recent studies are beginning to address these gaps.

<u>Origins and approaches of the fixed term task and finish AMR group</u>

4. Following discussions about AMR and responsible use of antimicrobials at FSA Board level, the FSA established a new fixed term 'Task and Finish' group, combining expertise from the existing ACMSF AMR group with additional co-opted experts, to reflect the wider range of expertise needed to address the following terms of reference.

   (i) *To identify research questions and potential approaches which would decrease uncertainty about any linkage between use of antimicrobials in food production, the incidence of antimicrobial resistance in pathogens and commensals in food production, and the growing AMR-related public health burden, and*

   (ii) *allow us to model the impacts of changes in use of antimicrobials in food production.* The terms of reference focussed on major food production animals i.e. poultry, sheep, cattle and pigs (identified as the main reservoir of AMR genes (ECDC/EFSA/EMA, 2017a), although it is acknowledged that antimicrobials are used in other food producing sectors, e.g. Game meat production and fish farming.

5. While this document makes reference to antimicrobials throughout, due to the fixed-term nature of this task and the comparative paucity of knowledge relating to other antimicrobials, the group has focussed its efforts on antibiotics and not included other antimicrobials within its report.

6. The group met in May, July, September, November and December 2017. Over that period, the group received evidence from key food animal sectors (pig, cattle (dairy and beef), sheep and poultry). The fish and gamebird sectors were not formally considered by the group. Given the fixed term nature of this task, the group focussed on identifying research priorities of specific significance to the FSA, rather than generating another comprehensive literature review of the expanding literature in this area. These priorities were based on evidence received from the above sectors, in combination with the group's expertise in relevant food sectors, to derive an up to date picture of the key AMR-related questions and challenges to the UK food chain.

7. In 2014 the Department of Health, Public Health England, Department for Environment Food & Rural Affairs, and Veterinary Medicines Directorate published an AMR systems map to provide a broad overview of the factors influencing the development of antimicrobial resistances and the interactions among these factors. The above AMR systems map was used in the development of a series of more detailed sub maps covering Animals and the Environment, Hospitals, G.P. Care & the Community and Pharmaceuticals, diagnostics and vaccinations.

8. The AMR task and finish group used the Animals and the Environmental sub map to help structure their considerations for the current work. The FSA provided a workshop for members of the task and finish group to validate and enhance the map in relation to the food chain (see Annex A). The group went on to produce a specific version of this map addressing the Task and Finish group's terms of reference. This Food Focused systems map (Annex B) identified eight main reservoirs for microbes/infection as the main areas to be considered by the group. These were:

- Pasture & Crops (addressed together)
- Amendments[2]
- Food Producing Animals
- Animal Feed
- Abattoir & Carcass Processing
- Food Processing
- Human Food
- Humans

9. As part of its work, the group has considered the evidence, or lack of evidence, concerning each of these areas, to inform identification of the most important knowledge gaps which could be targeted by further research or surveillance. Within these key areas, the group has developed a series of recommendations including those deemed to be of highest priority for the Food Standards Agency or for the FSA in collaboration with other departments or organisations.

10. In line with the terms of reference provided by Food Standards Agency, the group's recommendations are presented within a general framework which identifies:

- Areas the group consider to be strategically important to FSA, but where further work is not currently recommended as there is sufficient/good research and no obvious need for more work at this time.
- Areas the group consider to be strategically important to FSA, in which we are unaware of sufficient/good research, or current work by others. We suggest FSA should consider taking the lead in formulating and undertaking research in these priority areas.
- Areas the group consider to be of important to, but not necessarily the sole responsibility of, FSA. The group suggests that FSA could seek to

[2] The slurry pit reservoir marked on the systems map in Annex B has been substituted with the reservoir termed amendments by the group. Amendments refer to waste-derived materials that are spread onto land for some benefit (usually agricultural). For example, materials containing nitrogen, potassium or other nutrients may enhance crop growth, but can also contain chemical or biological contaminants.

encourage other relevant departments and agencies to take the lead in these areas. Where appropriate, FSA should consider collaborating with other relevant departments and agencies in these areas.

- Areas the group consider to be of lower/potential interest to FSA, but currently of lower priority, in which FSA should maintain a watching brief.

Antimicrobials in food production – the big picture

11. Antimicrobials have been used in animal husbandry for many years and are administered by various routes (directly, in feed, water) and varying scales of application with different objectives (individual, metaphylaxis, prophylaxis), mostly for animal health and welfare but also for production purposes. The use of antibiotics in animal feed as growth promoters has been banned in the EU since 2006 (EFSA-BIOHAZ, 2017).

12. It is difficult to estimate the precise quantity of antimicrobials used in food production globally, but the evidence suggests that it is at least as great as the amount used by humans. In some parts of the world antimicrobial use is far greater in animals than in humans; in the US, for instance, more than 70 percent of medically important antibiotics are used in animals (O'Neill, 2016).

13. In addition to the volume used, the types of antibiotics that are used in food production are important. Certain last-resort antibiotics for humans are currently being used extensively in animals, with no replacements in the pipeline. For example, the recent Chinese finding of a plasmid-encoded bacterial gene (*mcr-1*). This is a last-resort antibiotic for treating multidrug-resistant infections caused by Gram negative bacteria in humans. This resistance is of particular concern, as plasmid-encoded colistin resistance genes transfers easily among bacteria (O'Neill, 2016).

14. Until relatively recently, concerns about the development and dissemination of AMR bacteria and AMR genes have focused principally on the widespread use of antibiotics in human health, animal health and agriculture (WHO, 2016, Cahill *et al.*, 2017). Thus, the intermediate elements of the human food chain were viewed as “a conveyor belt” from farm to fork, where the main microbiological challenges were “quantitative” focusing on reducing the gross numbers of contaminating spoilage and pathogenic bacteria persisting in/on food products, with less emphasis on potential “qualitative” changes in the virulence or antimicrobial resistance of the relatively small numbers of surviving sublethally stressed bacteria in/on such food products.

15. Sublethal stresses, which damage (but do not kill) bacteria, or slow (but do not stop) bacterial growth, can trigger a range of bacterial stress responses which allow the bacteria to adapt to and survive within adverse conditions(Poole, 2012, Cohen,Lobritz and Collins, 2013). Unfortunately, these stress responses also activate undesirable mechanisms which encourage the development and persistence of AMR bacteria, and/or the exchange of genes, including AMR genes, between and among commensals and pathogens (Zhang *et al.*, 2000, Beaber,Hochhut and Waldor, 2004, Poole, 2012, Stecher *et al.*, 2012, Cohen,Lobritz and Collins, 2013, Nair *et al.*, 2013, Cohen, 2014, Cohen *et al.*, 2016, Fruci and Poole, 2016)

16. In overall terms, a number of stages of the wider human food chain are well recognised as providing stressful conditions within which AMR can emerge and be disseminated (AMR) genes(Cahill *et al.*, 2017), but much less is known about the AMR risks associated with bacterial stress responses within food processing. However, cursory examination of food processing environments and treatments confirms the presence/application of sublethal bacterial stresses which have (in

other clinical or industrial environments) been demonstrated to induce the development, and dissemination of AMR bacteria and AMR genes. Undesirable bacterially sublethal stresses of concern occurring within food processing include physical and mechanical stress/damage of bacterial DNA during food processing as reviewed by Gryson (2010) and Ceuppens (2014), and an expanding range of physical/chemical sublethal stresses which slow but do not prevent bacterial growth. These include: acidification, nonlethal heat treatments (Cirz and Romesberg, 2007, Foster, 2007); high pH, mild high/low temperature storage, osmotic stress and modified atmosphere packaging (McMahon *et al.*, 2007a, McMahon *et al.*, 2007b, Poole, 2012, Al-Nabulsi *et al.*, 2015, Van Meervenne *et al.*, 2015, Harms,Maisonneuve and Gerdes, 2016, Cahill *et al.*, 2017) contact with low concentrations of biocides, antiseptics, preservatives and metals (SCENIHR, 2009, Wales and Davies, 2015, Cahill *et al.*, 2017, Tezel and Pavlostathis, 2011)

17. Certain antibiotic classes are categorised by the World Health Organisation (WHO) as critically important antibiotics (CIA) for human use. These include the highest priority macrolides and ketolides, fluoroquinolones 3rd- and 4th-generation cephalosporins, polymixins and quinolones (WHO, 2017). Macrolides and ketolides are included in the highest priority of CIAs because they are known to select for macrolide-resistant *Campylobacter* spp. Macrolides are one of few available therapies for serious campylobacter infections, particularly in children, as quinolones are not recommended for treatment in children.

18. In December 2014, the European Medicines Agency (EMA) classed macrolides as category 1, which in practical terms means that the risk of their use in animals to public health is low or limited. Fluoroquinolones and 3rd- and 4th- generation cephalosporins were classified as category 2, which means the risk to public health is considered higher. This advice was subsequently updated to take into account new data on colistin resistance, and the expert group recommended that colistin was moved to category 2 (UK-VARSS, 2016). The classification of highest priority antimicrobials such as macrolides differs between the WHO and EMA classification systems. The EMA recommendations were developed to be tailored to the European region by taking the globally-focused WHO paper as a starting point, and subjecting it to evaluation by an expert panel of European veterinary and human health experts. The recommendations developed by that expert group took into account the scientific knowledge, epidemiology, regulatory landscape etc of the European region and are therefore more directly relevant to the UK than the globally-focused WHO recommendations.

19. The quantity of authorised veterinary antimicrobials sold throughout the UK has been reported to the VMD by pharmaceutical companies since 1993 and such reports have been a statutory requirement since 2005. Antimicrobial usage refers to the amount of antimicrobials purchased, prescribed and/or administered. The UK-VARSS 2016 report published by the VMD, for the first time included antimicrobial usage data from the pig, meat poultry, egg, gamebird and dairy industries, collected and provided on a voluntary basis (UK-VARSS, 2016).

20. The Government committed to reducing antimicrobial use in livestock and fish farmed intended for food to a multi-species average of 50 mg/kg by 2018, from 62 mg/kg in 2014. This target was achieved and exceeded two years early, with antimicrobial use in food-producing animal species decreasing by 27% to 45 mg/kg. In the animal sector, sales of highest priority CIAs declined in 2016 from an

already low level. Sales of 3rd/4th generation cephalosporins reduced by 12% to 0.15 mg/kg, fluoroquinolones by 29% to 0.24 mg/kg, and colistin by 83% to 0.02 mg/kg, i.e. considerably lower than the 1 mg/kg maximum target for colistin recommended by the EMA. High priority CIAs (fluoroquinolones, colistin and 3rd/4th generation cephalosporins) accounted for a small proportion of antimicrobial sales (<1%)(UK-VARSS, 2016).

21. There has been a notable reduction (24%) in macrolide sales (tonnes of active ingredient sold) between 2015 and 2016, although this does not necessarily reflect usage. Sales figures for macrolides between 2012 and 2016 in terms of tonnes of active ingredient sold were as follows: 2012 (41), 2013 (40), 2014 (48), 2015 (38), 2016 (29).

22. Usage of EMA classified HPCIAs in 2016 reduced by 73% in pigs, 78% in turkeys, broilers and ducks combined and 50% in dairy cattle compared with 2015. None of the datasets have 100% coverage, so may not be fully representative of the industry. This is particularly the case for pigs and dairy cattle where coverage is 62% and 33%, respectively (UK-VARSS, 2016); nonetheless these data are useful indicators of the UK picture. Commitment by industry to reduce and monitor the usage of antimicrobials is encouraging. Adoption and publication of 'The British Poultry Council (BPC) Antibiotic Stewardship scheme', the electronic Medicine Book for Pigs, 'eMB-pigs', and more recently, the report of the RUMA targets Task Force covering the main food-producing animal sectors demonstrate industry's engagement in reducing antimicrobial usage (RUMA, 2017).

23. Reducing antimicrobial use is only part of the issue. Whilst antimicrobial resistance can arise or be maintained as a consequence of any antimicrobial use, the way in which antimicrobials are used can affect the development of resistance profiles that are of importance to human health and animal health.

24. In this paper, the fixed term AMR Task and Finish group presents its considerations and recommendations, and highlights knowledge gaps for future targeted research and surveillance by the FSA and/or other departments and organisations. While the majority of the analyses and recommendations are presented in relation to the eight identified reservoirs, it is important to note that, very early in their discussions, the group agreed that there is a significant and long-standing lack of antimicrobial and AMR data in relation to UK-produced, processed and/or imported food, in absolute and comparative terms. For example, a systematic review conducted for FSA by the Royal Veterinary College, which focussed mostly on peer reviewed literature found a significant lack of AMR data at the retail level. In this area, we appear to have little readily available data and much less data than many other countries (Mateus *et al.*, 2016). While international surveys by ESFA, WHO, FAO, etc. can usefully highlight possible general risk trends in the development/dissemination /persistence of AMR in UK produced/processed foods, such reports also demonstrate significant heterogeneity in AMR types and prevalence among countries. Such heterogeneity may be due to variations in animal/plant species/strains and their animate and inanimate environments, as well as differences in food production and food processing practices. Data from other countries may suggest broad risk patterns, but cannot adequately inform decisions about antimicrobials and AMR within the UK food chain.

25. Apart from a small number of northern European countries which export considerable amounts of food to the UK, there is little or no data on AMR in foods imported into the UK. Brexit-related changes in the relative amounts of foods imported from non-EU countries are likely to change the qualitative and quantitative antimicrobial and AMR related challenges in foods consumed in the UK.

26. Scientists always “want more data”. Nevertheless, in this case, considerably more data will be required if we are to understand and reduce the risks to human health posed using antimicrobials and the presence of AMR pathogens and commensals in domestically produced/processed food, and in imported foods. This area should be a significant priority for FSA, bearing in mind its importance in the activities and responsibilities of FSA. More systematic food specific work is needed, much of which is unlikely to be undertaken by other agencies.

27. **High priority recommendation - UK standardised methods should be developed and agreed for co-ordinated, regular, targeted surveillance, detection, identification, quantification, description and subsequent reporting/archiving of antimicrobial resistance/susceptibility at appropriate stages of the UK food production /importation /processing/retail chains. These processes should be effectively integrated with environmental, veterinary and clinical surveillance systems and agencies, in line with “One Health” principles, but FSA will have to be fully engaged in the food specific aspects of this work if the aims of ToR (i&ii) are to be achieved.**

CHALLENGES IDENTIFIED WITHIN THE FOOD FOCUSED RESERVOIRS

Crops and Pastures

Background

28. Crops and pastures represent a potential route by which AMR pathogens, or reservoir organisms with mobilisable antibiotic resistance genes (ARGs) that could pass their genes to pathogens, could potentially enter the human food chain. The direct risks to consider are via vegetable crops, including leaf, root and possibly grain crops as well from grazing animals, including lamb and beef. There may be risks of transmission through wildlife and consumption of game meat. There is also an indirect risk through the transfer (run off) of soil and spread materials into adjacent water courses and hence onwards to niche crops such as water cress, as well as to fish and shellfish. Most of these risks stem from the spreading of materials to land which includes outputs from the "slurry" component that was identified in the AMR systems map. Figure 1 shows compartments associated with crops and pasture where AMR might spread/be selected, and main flows between these compartments.

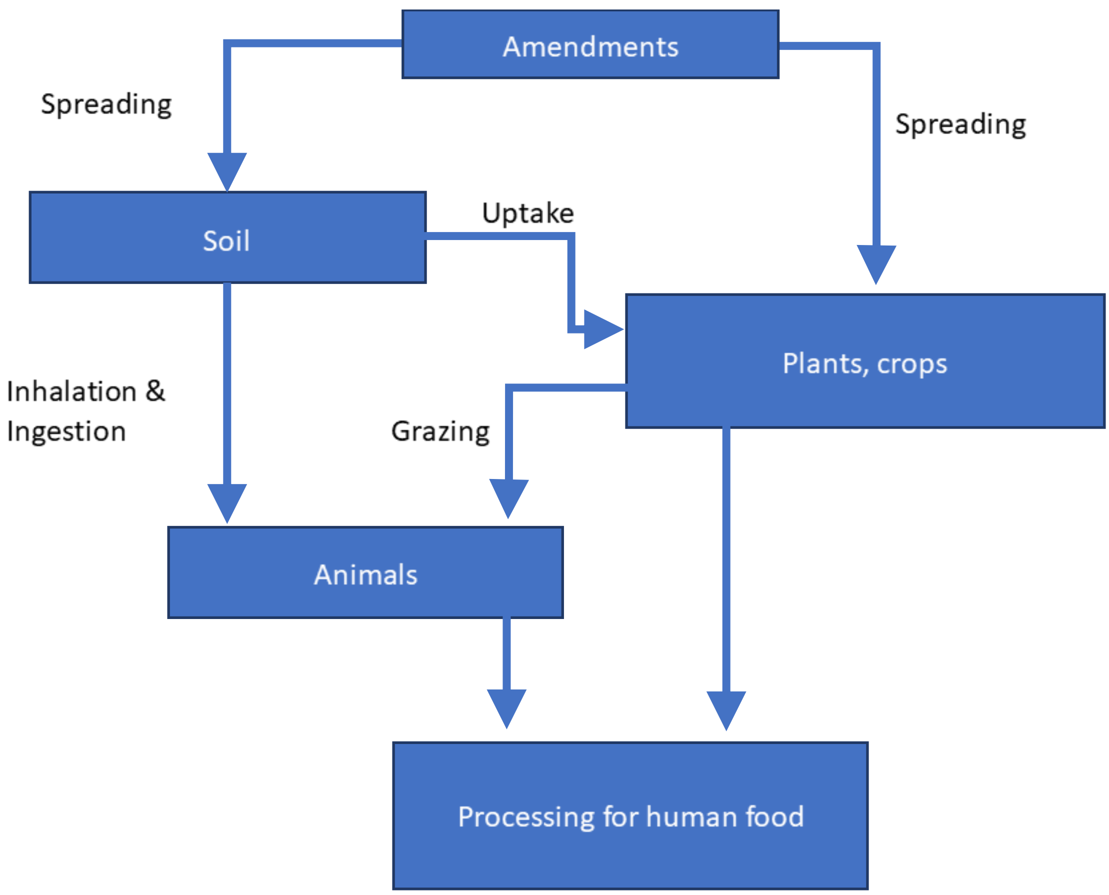

Figure 1. Compartments associated with crops and pasture where AMR might spread or selected.

Amendments

29. Amendments refer to waste-derived materials that are spread onto land for some benefit (usually agricultural). For example, materials containing nitrogen, potassium or other nutrients may enhance crop growth, but can also contain chemical or biological contaminants. The Environment Agency has identified a range of amendments that could potentially spread antimicrobial resistance onto fields:

- Farm wastes such as slurry from animal husbandry. This may include both 'solid' manure and liquid slurry, which pose different risks and have different regulations, e.g. in nitrate vulnerable zones (NVZs). These wastes may be spread onto land other than that of the farm where they were created, particularly in the case of high intensity farms where animals are housed and there is limited surrounding arable land.

- Final residues from sewage treatment works (STW) that are spread to land. Sewage works receive waste from humans undergoing treatment with antibiotics, from hospitals and clinics, from some farms and potentially from any manufacturing sites. Sludges may be treated to kill pathogens before spreading occurs (for example using heat or pH adjustment) but there is little information on the effects of different wastewater treatments on AMR or the subsequent sludge treatments.

- Non-mains sewage that is discharged to soakaway or leaks from septic tanks. Generally, these are small, but in rural areas can be a significant proportion of the total sewage system.

- Other wastes, including industrial and household waste residues spread for example following anaerobic digestion and driven, in part, by the desire to decrease the use of final disposal to landfill.

30. A key issue for spread materials may be the potential for combinations of substances to promote co-selection for AMR, for example due to presence of biocide residues or heavy metals.

Areas which are important but where there is sufficient/good research and no obvious need for more work

- Research in this area is generally quite limited, especially in a UK context, as many studies have been conducted overseas. While research is generally high quality, it is impossible to point to areas in which there has already been sufficient research.

Areas which are important but we are unaware of others doing work on at present and so contain research gaps that the FSA could consider as priority.

Uptake of AMR pathogens or antimicrobial resistant genes (ARGs) from amended soils into crops, grazing or game animals

31. Current evidence suggests that there is transfer of resistance genes from soil amendments to vegetables (Wang *et al.*, 2015, Tien *et al.*, 2017), although the degree of that transfer might be low (Marti *et al.*, 2013, Lau *et al.*, 2017). Moreover, some bacteria, e.g. *E. coli*, die off on leaf crops. (McKellar *et al.*, 2014) for example, modelled the death rate of bacteria on leaf crops. Therefore, it might be relevant for FSA to be more active in promoting research, especially if this can help promote improved practise and decrease risks. It will be important to consider the diversity of factors that are likely to influence risk, i.e. The nature of the amendment, what it contains in relation to animal husbandry, soil type and composition (Blau *et al.*, 2017), manure treatment (Tien *et al.*, 2017), application method (Hodgson *et al.*, 2016), history of previous amendments, local climate and weather, field management practises, crop type, crop handling practises. Since these factors are likely to vary widely, and none of the studies cited were based in the UK, research conducted into crops and fields under UK conditions could be of value. The role of wildlife in the spread of AMR (Arnold,Williams and Bennett, 2016) is also largely unexplored, with potential consequences for game meat. Hence, to assess the scale of risks to humans we need to know much more about the different sources and pathways that each crop and uptake route represent. Specific questions include:

- Which plants or crops are important and why? Is the main concern over leaf or root crops that are eaten raw (e.g. lettuce or radish), or over contaminated soil on crops that may be cooked (e.g. potato)?
- To what extent are different resistant organisms taken up by plants?
- What organisms are more important – pathogens or reservoirs?
- How long do AMR organisms survive on plants?
- What is the most important route of uptake by grazing animals (from ingestion of plants, soil or faecal matter)?
- How important is the role of wildlife in the spread of AMR?
- What are the risks to game?

32. FSA could consider building a quantitative risk model that can consider propagation of risk from amendments to soil to crops to processing to eating. Such a model could be used to identify critical control points for relevant intervention and advice.

Areas which are important, but not necessarily for FSA to address, but for other departments

Does amendment lead to spread or selection in AMR in soils?

33. Many studies show that manure (e.g. (Marti *et al.*, 2013, Cook,Netthisinghe and Gilfillen, 2014, Fahrenfeld *et al.*, 2014, Muurinen *et al.*, 2017)) and sewage (e.g. (Chen *et al.*, 2016)) amendments lead to increased AMR in the soil. This resistance decreases with time when amendment stops (Marti *et al.*, 2014, Muurinen *et al.*, 2017), partly due to limited survival of *E. coli* and other faecal bacteria in soil (reviewed in (Ongeng *et al.*, 2014)). A key question lies around the relative

importance of AMR pathogens, reservoir organisms, mobilisable resistance genes and selective agents. Although evidence is incomplete, the most significant of these is probably mobilizable elements under co-selective pressure. A recent study on pig manure amendment (Chen *et al.*, 2017) has shown that it is the microbial rather than chemical components of manure that are responsible for spread of AMR into soil. One study has indicated that faecal *Clostridium* spp. and soil *Acinetobacter* and Pseudomonas spp. are likely to be important for spread of resistance genes (Leclercq *et al.*, 2016). Moreover, heavy metal contamination is likely to be a co-selective agent leading to increased persistence of AMR(Song *et al.*, 2017).

34. The Environment Agency (E-A, 2017) regulate the quality of spread materials using a range of soil screening values as the recovery of wastes to land under the Environmental Permitting Regulations 2010. These regulations aim to ensure that potential agronomic and economic benefits are balanced against the broader health and environmental risks including to the microbial functioning of the soil. This includes setting screening values for wastes containing substances (e.g. biocides) that may potentially affect the persistence of resistance in agricultural soil. Most of this work predates the recognition of AMR as a risk beyond the clinic or animal house and significant gaps in knowledge remain.

35. The FSA should keep abreast of current research in the area, but further research is likely to be out of scope of the FSA itself. Important questions around what happens to AMR after soil amendment include:

- To what extent does AMR spread from amending communities to soil communities?
- To what extent is spread of AMR due to population changes in soil microbiota following amendment?
- Which organisms are important either as potential pathogens or as reservoirs?

Areas which are of interest but of lower priority

36. Impact of AMR in crops and fields on grazing or game animals might be considered lower risk than the impact on vegetable crops, so might be considered lower priority. There is, however, insufficient evidence to strongly support this.

High priority Recommendations

37. FSA should consider the risks associated with vegetable crops, especially leaf and root crops. The FSA could support research to identify high risk crops and quantify those risks, including:

- What are the AMR risks associated with leaf, fruit, or root crops that are eaten raw?
- What are the AMR risks associated with soil entering kitchens from root crops that may be eaten cooked?

Animal Feed

38. The AMR bacterial infections in animals that are of high risk to human health are likely to be zoonotic pathogens transmitted through food, such as *Salmonella* spp., *E. coli* and *Campylobacter* spp. In addition, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA MRSA) and extended spectrum beta-lactamase-producing *E. coli* (ESBL *E. coli*) are emerging global issues. The sources of such AMR pathogens can be multiple, but animal feed has been identified as an important reservoir. Furthermore, animal feed may be supplemented with antimicrobials and this may influence the emergence and maintenance of AMR. Therefore, there is an urgent requirement to better understand the role of animal feed in the emergence and maintenance of AMR in animals, and to investigate novel mitigation strategies.

39. **Gaps identified**

- Additional research is needed to develop reliable alternatives to antimicrobials in animal feeds. Research in this area should include the identification of new alternatives, understanding their modes of action and identifying any co-selection risks associated with their use.
- There is a need for a greater understanding regarding the drivers of AMR in animal feed (residues and resistance in bacteria). Regular targeted surveillance of specific feed items for AMR is therefore essential.
- The influence of feed production processes – decontamination measures, storage, chilling, freezing, etc. on AMR has not been systematically assessed.
- The risk of heavy metals in animal feed and the influence on AMR remains relatively unquantified.
- Risk ranking of animal feed (both European and non-European) in relation to AMR should be considered.
- There is a paucity of knowledge regarding the microbiological safety and AMR risk associated with raw feeds for companion animals.
- Cross contamination of animal feed with antimicrobial residues at the feed mill needs further investigation.

40. **Recommendations:**

- Further research on the use of heavy metals in animal feed and the influence on AMR are urgently required.
- Better legislation regarding antimicrobial residues in animal feeds.
- Scanning surveillance of animal feeds for AMR.

- Better understanding of the transmission of AMR in animal feed matrices.
- Companion animal feeds need to be assessed – particularly with the popularity of raw feed for dogs and cats.

Areas where some studies have been undertaken, but further research is required

41. Heavy metals in animal feeds

- Heavy metals, such as copper, are increasingly supplemented in pig diets as an alternative to antimicrobials to promote growth. Common gut commensal bacteria may acquire plasmid-borne, transferable copper resistance (*tcr*B) gene-mediated resistance to copper. The plasmids harbouring the metal resistance also often carry genes conferring resistance to tetracyclines and macrolides. The potential genetic link between copper and AMR suggests that copper supplementation may exert a selection pressure for AMR. A recent study suggests that supplementing with copper or antimicrobials alone does not increase copper-resistant enterococci; on the other hand, supplementing antimicrobials with copper increases the prevalence of the *tcr*B gene among faecal enterococci in piglets (Amachawadi *et al.*, 2015).

- Dietary zinc oxide has been used in pig nutrition to combat post weaning diarrhoea. Recent data suggest that high doses (2.5 g/kg feed) increase bacterial AMR development in weaned pigs. Recent studies have investigated the prevalence of AMR genes in the intestinal tract of weaned pigs (Vahjen *et al.*, 2015). The studies demonstrated that the copy numbers for tetracycline and sulfonamide resistance genes were significantly increased in the high zinc treatment compared to the low (*tet*A: p value $< 10^{(-6)}$; *sul1*: p value $= 1 \times 10^{(-5)}$) or intermediate (*tet*A: $P < 1.6 \times 10^{(-4)}$; sul1: $P = 3.2 \times 10^{(-4)}$) zinc treatment. Regarding the time dependent development, no treatment effects were seen one week after weaning, but significant differences between high and low/intermediate zinc treatments evolved two weeks after weaning. The increased number of *tet*A and *sul*1 gene copies was not confined to the hind gut, but was already present in stomach contents (Vahjen *et al.*, 2015).

High priority recommendations where further research is urgently required

42. Alternatives to antimicrobials in animal feed

- The rapid emergence of multidrug-resistant (MDR) pathogens and increased regulations regarding the use of growth promoters coupled with a rise in consumer demand for meat products has prompted the search for alternative antibacterial agents for use in food animals. Antimicrobial peptides (AMPs), produced by bacteria, insects, amphibians and mammals, as well as by chemical synthesis, are possible candidates for the design of new antimicrobial agents because of their natural antimicrobial properties and a low propensity for development of AMR by microorganisms (Wang *et al.*, 2016). Other alternatives include probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics, antimicrobial peptides, hyperimmune egg antibodies, metals and bacteriophages. Although the beneficial effects of many of the alternatives developed have been well demonstrated, often these products lack consistency and thus efficacy can vary. Furthermore, their mode of action needs to be better defined (Gadde *et al.*, 2017). Any possible effects relating to the use of these alternatives to antimicrobials in animal feed and any unintended effects relating to co-selection and transmission of AMR genes in animals resulting from such usage also needs to be explored.

43. Companion animal raw feeds

- Feeding companion animals (cats and dogs) raw meat-based diets (RMBDs) is commonly practiced by many companion animal owners and has received increasing attention in recent years. It may be beneficial for the animals. However, there are concerns that such practices may pose a health risk for both pets and their owners, as RMBDs may be contaminated by enteric pathogens such as *Campylobacter* spp. *E. coli*, *Yersinia* spp. and *Salmonella* spp. all of which are zoonotic bacteria causing enteritis not only in humans but also in companion animals. Further research is required on the prevalence of these pathogens in companion animal food and the contribution to human AMR that these pathogens may make. A recent study showed that such pathogens (*Campylobacter* spp., pathogenic *E. coli*, *Yersinia* spp. and *Salmonella* spp.) were not recovered by standard culture, indicating a low contamination level in pre-frozen RMBDs (Fredriksson-Ahomaa *et al.*, 2017).

44. **The following is lower priority, but still of interest**

Cross contamination of feed

- The cross-contamination of non-medicated feed with residues of antimicrobials is an animal and public health concern associated with the potential for the selection and dissemination of AMR in commensal and zoonotic bacteria. It is hard to reduce the risk to zero as it is the result of factors occurring at different levels. The use of antimicrobial-medicated feed should therefore be avoided as much as possible to reduce selection (Filippitzi *et al.*, 2016).

45. Contamination of waste raw milk with antimicrobials following the treatment of dairy cows (milk from these cows is frequently fed to calves) should be carefully evaluated for a range of antimicrobials, particularly CIAs such as 3rd- and 4th-generation cephalosporins (EFSA-BIOHAZ, 2017).

Food producing animals

Background

46. The mapping exercise (Annex A and B) identified the importance of food producing animals as one potential reservoir of AMR in the food chain, among others. Potential pathways for transmission of AMR bacteria from this reservoir to humans include via food production routes (slaughter, milk or eggs), but also *via* direct human occupational contact or via waste and the environment.

47. The group identified generic factors that might drive emergence of AMR in food animal and farmed fish production systems, but also factors that might drive further dissemination of AMR within the farm environment. In summary, these factors were categorised to include (i) husbandry factors (including stockmanship, livestock housing systems supporting continuous flow versus all-in all-out populations, cleaning and disinfection, and fish production systems), (ii) biosecurity standards limiting entry of AMR microbes via soil, water, other livestock, pets and wildlife including invertebrates, and finally (iii) veterinary disease treatment and prevention strategies including antimicrobial choice, administration methods and other disease prevention strategies including vaccination. The possibility of two-directional flow of genetic AMR determinants between pathogens and commensal organisms at animal, fish or farm level was also noted.

48. Better and more detailed evidence is needed about how changes in antibiotic use at production animal or fish level might ultimately impact on AMR at the human level. This could focus on understanding the causal relationships between antibiotic usage at animal or fish level with the prevalence and diversity of AMR in those same populations, but also with AMR prevalence and diversity at human in-contact level and, likely quite separately, human consumer level. The availability of evidence for these causal links would help not only in the development of predictive models, but would also allow prioritisation of specific pathogens for additional surveillance, specific preventive activities at farm level and the targeting of specific antibiotics.

49. AMR exchange between animals and in-contact humans is likely to be relatively straightforward, while the transmission and patterns of AMR transfer from living food-producing animals or fish to consumers via the food chain may be significantly affected by spread, cross-contamination or environmental persistence in the abattoir, processing environment and beyond. Understanding the processing environment, the processes involved and the influence these factors have on prevalence and diversity of AMR in food (compared with in the original food producing animals) is therefore integrally linked with understanding and managing how AMR is optimally addressed in live animals as part of the wider picture.

50. The remainder of this section attempts to identity areas where there is existing sufficiency or insufficiency of evidence to support these goals.

51. **Areas where there is sufficient evidence:**

- There are few examples of sufficient evidence in the current context. Indeed, much evidence remains to be gathered. However, it is worth noting that much of the existing literature on the prevalence and diversity of AMR bacteria at livestock or fish level was (1) not obtained using standardised methodologies, especially in terms of cut-off values for resistance, or (2) not focussed on bacteria that are not agents of food borne zoonoses or food related indicator organisms. Arguably, there is a sufficiency of such studies that do not use standardised methodology, or that do not apply it to prioritised bacteria associated with food-borne infections in humans, or that do not demonstrate associations between prevalence of AMR bacteria in living food producing animals with changes in usage level of antibiotics in the same population.

52. **High priority recommendations:**

Five high priority recommendations are identified in this section:

1. Large scale surveillance studies at 'macro' level to identify linkages and generate risk-based priorities for more detailed research are needed. Robust insights on linkages between antimicrobial usage in livestock and fish, and prevalence of AMR in food animal bacteria and human consumers of resulting 'products of animal origin' depend on large scale standardised surveillance. Such surveillance should also take account of key contributory factors including human antimicrobial consumption and whether/how the prevalence and diversity of AMR bacteria changes from the food producing animal on the farm or fish farm, during transport and in the abattoir or at processing (including mixing of animals and environmental contamination of transport, lairage and abattoir processing equipment and practice), and beyond.
   The 2017 JIACRA report (ECDC/EFSA/EMA, 2017a) provides preliminary valuable insights into this question[3]. The report highlights the existence of such linkages but notes that these are not consistent across all the key food borne bacteria, nor across the range of prioritised antimicrobials considered. Resources may therefore be well placed in optimising the quality of surveillance so that research questions can be better framed. Areas of improvement in surveillance inputs include standardisation of input data parameters (antimicrobial panels included, units of antimicrobial consumption, definitions of resistance based on epidemiological cut-off values (ECOFF) and methodology of analysis). Furthermore, an increase in the granularity of surveillance data linked to each isolate, for example recording human clinical context or livestock production context could assist in deeper analysis, including association with risk factors - thereby informing potential interventions.

   Another important opportunity exists for collection of more detailed data on the formulation of antibiotics employed; noting that prolonged action preparations probably have different pharmacokinetic properties. Similar observations were noted in the FSA's 2016 '*Systematic review to assess the significance of the food chain in the context of antimicrobial resistance'* (Mateus *et al.*, 2016). Outputs from extensive surveillance such as that described by JIACRA only allow the identification of statistically significant linkages, rather than causality, but hypotheses can be generated and ranked in a risk-based manner for testing through discrete research approaches.

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[3] *ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals"* (http://www.efsa.europa.eu/en/efsajournal/pub/4872)

The design of such large-scale surveillance studies also requires agreement on a manageable prioritised list of bacteria for inclusion. Preliminary meta-analysis of published literature would support identification of those bacteria (commensal and pathogenic) of livestock that might contribute to food-borne AMR bacteria in humans. The WHO has published a global priority list of antimicrobial-resistant bacteria to guide research, discovery and development of new antibiotics (WHO, 2017). This list includes bacteria with a potential food-borne zoonotic path of transmission, including Enterobacteriaceae, *Campylobacter* and *Salmonella* but meta-analysis may reveal other candidate organisms.

More recently, a paper was published by EMA, EFSA and ECDC (ECDC/EFSA/EMA, 2017b) which, while the paper states that the indicators are for the most part not suitable to monitor effects of targeted interventions in a specific sector, may (if widely adopted) generate a more harmonised data set in the medium term.

2. Detailed exploration of linkage (from above) between antimicrobials, animal or fish bacteria, and human pathogens is required. This would potentially include comprehensive understanding of the genetic basis for antimicrobial resistance in key food-borne pathogens using very large collections of well-phenotyped collections for genome wide association studies and studies of movement of traits across phylogenies and over time. The availability of this whole genome sequence data for very large collections of isolates of key bacterial species, with consistently phenotyped AMR profiles, built up over time and including geographic, human, animal, disease associated/non-disease associated information will assist more targeted investigation of the identified linkages and the factors underpinning them (Woolhouse *et al.*, 2015). In this way, models describing the impact of changes in antimicrobials use in food producing livestock or fish on AMR in humans might be generated at a more specific and therefore informative level (specific in terms of livestock species, pathogen species, antimicrobial class and pharmacokinetics of relevant preparations). These more specific data might also enable better targeted interventions.

3. The development of models, identification of risk factors and feasible interventions for those specific situations where there are defined linkages between usage of specific antimicrobials in food producing animals or fish with AMR in the human consumer of products of animal origin. Such models must account quantitatively for the multiple pathways by which humans are exposed to AMR-carrying bacteria, beyond products of animal origin. An example of such a model which already exists for *Salmonella* spp. (not AMR *Salmonella* spp.) was developed by the Veterinary Laboratories Agency in 2011 with funding from EFSA (EFSA, 2010). This model has in the past formed an important part of UK evaluation of the risk posed by the colistin resistance (*mcr*-1) to consumers of food from pigs on affected farms. Development of risk assessments covering AMR aspects would enhance the confidence with which consumer safety under similar future circumstances can be evaluated.

4. Social science-based understanding of the factors influencing antimicrobials use in food animal production, including value-chain analysis, farm assurance schemes, regulatory landscape, defined impacts on animal welfare and consumer understanding.

5. Economic analysis of effects of AMR-related interventions on-farm.

Areas of interest but of lower priority for FSA (high priority for others):

- Detailed comprehensive understanding of the genetic and transcriptional basis for AMR, class by class, including co-selection, for prioritised bacteria. This includes better understanding of the impact of different formulations, for example prolonged action preparations, with likely different pharmacokinetic features within the same class of antimicrobials.
- Better understanding of the factors governing diversity and the dynamics of flow of AMR capability between commensal and pathogenic bacteria, and also unculturable bacteria.

Abattoir and Carcass processing

Background

53. The slaughter point is a major issue with regard to direct animal to human contact and contact with effluents from the animal. It appears that the health of the workers in this environment is recognised to be at risk and has been studied for other health and disease issues yet has not been specifically targeted for AMR transmission events albeit there are a number of studies on MRSA, yet often these focus on animals and food rather than the interaction between animals and humans (Moon *et al.*, 2015, Normanno *et al.*, 2015). There is a societal responsibility to look at these processes more carefully and to understand how these workers are protected and how these workers are subsequently managed if they enter a human healthcare setting.

54. At this stage, no routes should be excluded, evidence needs to be developed to piece together where there are gaps.

55. There is an annual throughput of approximately 2.6 million cattle, 10 million pigs, 14.5 million sheep and lambs, 80 million fish and 950 million birds by >313 abattoirs in the UK. Abattoir and carcass processing are in general strongly perceived as opportunities for the spread of intestinal and skin organisms between animals. This may include organisms with genes encoding for antibiotic resistance. Logically the people involved in slaughtering and processing meat are one of the high-risk groups for AMR transmission from animals to humans. While not directly within the scope of this paper, appropriate Government Agencies need to work to understand this risk and if found to be higher for such workers develop methods to manage this risk.

56. Routes of dissemination include aerosol dispersal and surface-surface contact. Aerosol dispersal being more difficult to limit and control than surface-surface contact. The resulting dissemination can lead to cross-contamination of carcasses.

57. Abattoir & carcass handling personnel being directly exposed to skin and intestinal organisms. Abattoir and carcass handling personnel can transfer organisms between carcasses by direct handling. There are unknown consequences for AMR transmission.

58. Influences on carcass contamination level include pre-slaughter faecal load, transit and holding times.

59. Control measures include visual inspection for cleanliness, biocide usage, washing hides, microbiological sampling plans, as well as pathogen specific bovine tuberculosis screening for which there are eight approved reactor slaughterhouses.

60. Healthcare of the abattoir and processing workers with their levels of illness and likelihood of entering a human healthcare facility.

Key issues of concern

61. There are two issues with respect to the abattoir and carcass processing step in the food system which are of concern regarding the transmission of organisms with genes encoding for AMR.

1. The contamination of the meat and meat products with organisms having genes encoding for AMR.

- This is a well-researched area in terms of considering the transmission of food borne pathogens, irrespective of AMR traits. There have been a number of risk analyses undertaken that could be extended to quantify the risk of contamination of meat and meat products by organisms with genes encoding for AMR.

- Transit to the abattoir is a stage whereby bacteria of concern can be dispersed from a contaminated animal to a larger number of previously 'clean' animals. Therefore, current estimates of AMR carriage by meat carcasses do not necessarily reflect those of live animals, and could vary depending upon abattoir practices.

- Indicator *E. coli* and *Campylobacter* spp. isolates are currently obtained through active monitoring programmes, which are based on representative random sampling of carcasses of healthy animals at the slaughterhouse. For *Salmonella* spp. isolates from broilers, laying hens and fattening turkeys, included those which originate from *Salmonella* national control plans, as well as isolates from carcases of broilers and fattening turkeys, sampled as part of process hygiene criteria. The amount of cross-contamination by AMR organisms from the skin (i.e. *Staphylococcus aureus*) and from intestinal contents (i.e. *Enterococcus faecalis, E. coli, Salmonella* serovars & *Campylobacter* spp.) may differ and the contribution from either is uncertain. The bacteria present may be influenced by the slaughter processes (exposure to biocides etc.) which will influence bacterial survival.

- The limitations of sampling plans are not always recognised, and may be due to financial limitations rather than representative analysis. For example, the temporal trend of MRSA in animals was assessed in Switzerland for fattening pigs at slaughter using nasal swabs from 2009 to 2015. The method used involved sampling one pig per herd at slaughter. This could be imprecise since pigs can be intermittently colonised and because sampling at slaughter can be influenced by colonisation of animals in the abattoir lairage (Bangerter *et al.*, 2016, ECDC/EFSA/EMA, 2017a).

2. The transmission of AMR organisms to the abattoir and processing workers.

- The sources of bacteria on post-slaughter meat will be from the animals from which the meat was derived, the abattoir workers, and the environment in which meat was prepared and stored. The direct transmission to workers is less well documented and needs more thought with regards to the procedures to (i) protect the health of these workers and (ii) the monitoring of their general health and likelihood to seek healthcare when sick.

62. While direct dissemination between animal-animal and animal-human is well recognised, further research could be considered to reduce the exposure levels. The level of intestinal organisms of concern can be modified through pre-slaughter practices and therefore could be a route of reducing subsequent dispersal. At this stage, no routes should be excluded, evidence needs to be developed to piece together where there are gaps. This area is important and we are unaware of others doing work on at present and therefore FSA should consider this as a priority.

High priority recommendations

63. FSA should work with other departments responsible for occupational health to ensure there is further investigation relating to the direct transmission of AMR to slaughterhouse workers. Further research is required on:

- Overall risk levels of transmission from animals to workers in this environment separated into species groupings and types of slaughter plants
- Procedures to protect health of these workers and the monitoring of their general health
- Research on the likelihood of the workers seeking healthcare when sick and how they are managed in terms of risk.

Important areas that are well covered

64. The area of contamination of meat and products is well researched in terms of risks of foodborne pathogens through a number of risk analyses. Additional research could be useful in the application of this research to quantifying risk of AMR. Abattoir and carcass processing is commonly perceived as a stage whereby bacteria of concern can be dispersed from a contaminated animal to a larger number of previously ‘clean’ animals. Further research could be useful in terms of looking at dispersal of AMR at this stage of the food system.

Areas of interest and of lower priority

65. In the scope of this study, the organisms of concern are those with genes encoding resistance to CIAs. The volume of cattle, pig, sheep and poultry passing through slaughter houses in the UK is considerable and results in generic control of dispersal through aerosols and surface-surface contact. While direct dissemination animal-animal and animal-human is well recognised, further research could be considered to reduce the exposure levels. The level of intestinal organisms of concern can be modified through pre-slaughter practices and therefore could be a route of reducing subsequent dispersal. This area is proposed as warranting further consideration.

Secondary food processing

66. Secondary food processing activities include post-harvest/slaughter removal of damaged or undesirable/inedible elements, portioning and preparing the raw material, e.g. deboning, portioning and forming, as well as value modification (marination, fermentation), mixing with other edible materials, including processing aids, decontamination treatments (including heat treatment), and stabilising by chilling/freezing /MAP/packaging.

67. The nature of the qualitative and quantitative microflora of most raw food entering secondary processing, and the antimicrobial resistant bacteria/genes present within this microflora, are dictated by the outcome of multiple interactions with significant animate and inanimate elements of the production environment, i.e. other animals, soil, water, feed, humans and husbandry /veterinary practices.

68. During secondary processing, poor hygiene practices allow this microflora to cross contaminate and re-contaminate food product. Such contamination is more likely when operative hygienic practices are inadequate or absent, and many food safety/standards agencies (including FSA) have been very active in this area for many years, with the general aim of improving operative hygiene protocols to reduce overall levels of bacterial contamination in food products. In relation to AMR, enhanced operative knowledge and practice may be necessary to reduce the additional risks associated with handling (and cross contamination from) AMR contaminated food. For example, current classifications of relative food safety of Food Businesses (FBs)/food types/processes may change if AMR specific risks become more important.

69. **Priority rating: This broad area should remain of importance to the activities and responsibilities of FSA. However, apart from possible recalibration of foodborne risk assessment/management to recognise emerging AMR specific risks, further specific AMR related research in this area may be of lower priority to FSA.**

70. **Recommendation - FSA should continue to work in collaboration with other agencies to review and where necessary enhance operative/company knowledge and hygienic practices to reduce cross contamination with AMR bacteria/genes within the UK secondary processing chain.**

71. Relatively recent changes in secondary food processing/storage objectives and methods may encourage the endogenous development/dissemination of undesirable AMR genes and gene combinations. Most antibacterial processes within secondary food processing used to be primarily bactericidal, i.e. “simple, short, sharp, and persistent”, aiming to permanently inactivate most of the bacteria in the treated food. This approach extends food safety and stability beyond the treatment period, although such improved food safety can come at the cost of damage to product quality.

72. More recently, there has been a sustained movement away from bactericidal (lethal) processes toward milder bacteriostatic (inhibitory) processes which can leave considerable populations of stress inhibited/damaged bacteria in treated

foods. These sub-lethally stressed bacteria can recover and grow if the bacteriostatic stresses are reduced or removed.

73. Bacteriostatic treatments are more complex, involving milder/minimal processing (causing less damage to product quality), but requiring precise and longer-term control of a complex set of environmental conditions, to effectively suppress bacterial growth and metabolism throughout the product shelf life. They typically involve multiple hurdle technologies (selecting and simultaneously applying a number of factors or treatments (e.g. mild temperature changes, acidification, osmotic stress, fermentation dehydration, MAP, “mild” pasteurisation /minimal processing, along with low concentrations of inhibitory agents (e.g. essential oils). In such processes, food safety and quality depend on consistent application of **all** of the hurdles, **all** of the time, to adequately suppress the repair/growth of stress damaged /inhibited/slow growing bacteria in food products. Failure or interruption of any one of the above hurdles may allow slow bacterial growth under sublethal/sub inhibitory conditions and increase the risks of the development and dissemination AMR genes within a food product, especially if the overall process does not include a control step which eliminates significant pathogens.

74. It is becoming increasingly clear that such sublethal stress conditions trigger bacterial defence/repair systems and significantly increase the “genomic plasticity” of stressed bacteria (Poole, 2012, Cohen, 2014, Fruci and Poole, 2016). Thus, individual damaged cells are less efficient in repairing randomly occurring mutations (losing individual genetic veracity), and stressed/damaged bacterial populations are more likely to release or absorb genetic material among and between bacterial species and strains (losing population homogeneity). These individual and population level stress responses mean that, in foods stored or processed under inadequate bacteriostatic conditions, sub-lethally damaged bacterial populations constitute “hot spots” in the development and dissemination of AMR.

75. **Priority rating: This area is very important, and should be a high priority for FSA, as the lead organisation in relation to food processing and safety. Despite the wider recognition of the adverse AMR potential of bacterial stress responses in other environments (e.g. health care/agriculture/water management), relatively little is known about the AMR impact of the use of bacteriostatic treatments during food processing in food processing (re ToR(i). A specific review of the impact of such treatments is necessary in modelling (and informing industry in reducing) the impacts of changes in “milder” bacteriostatic food processing technologies during food processing and storage (re ToR(ii).**

76. **High priority recommendation- As the lead organisation in relation to food processing and safety, FSA should commission a research review on the impact of currently used sub-lethal food processing technologies in encouraging the emergence, persistence and dissemination of AMR genes within secondary food processing activities.**

77. Some novel/emerging **food contact surface** decontamination technologies have the potential to apply sub-lethal stress to bacteria on food processing equipment (with the consequences outlined above). For example, low temperature plasma, and other means of generating highly reactive oxygen species, have been specifically demonstrated to increase the rates of development /persistence of mutations in bacteria. Thus, low temperature plasma treatments are used to generate possibly desirable mutants in lactobacilli and other useful bacteria (Zhang *et al.*, 2014), and are also used in the decontamination of food contact surfaces (Trevisani *et al.*, 2017). While such mutation generating technologies are valuable in some circumstances, it would be prudent to explore the potential impact of the wider application of such technologies in accelerating the rates of generation/persistence of mutations in pathogens and commensals on secondary food processing surfaces.

78. **Priority rating: This area is probably of interest, but lower immediate specific priority, to FSA. Thus, inanimate surfaces within food processing environments share many characteristics and challenges with inanimate clinical and environmental surfaces. While novel and emerging techniques for decontamination of food contact surfaces may become of increasing interest to FSA, an initial review of wider studies of the AMR related aspects of new and emerging surface decontamination technologies would be an appropriate first step in addressing ToR (i&ii).**

79. **Recommendation - FSA, as the lead organisation in relation to food processing and safety, should consider commissioning a review to reduce the knowledge gap on the impact of emerging/novel sub-lethal food contact surface decontamination activities and conditions in encouraging the emergence/persistence of AMR on such surfaces.**

80. In both of the above cases – i.e. within **food** and **on food contact surfaces**, it would be appropriate to focus (at least in the first instance) on higher risk material, i.e. involving raw materials which are already recognised to carry undesirable/zoonotic bacteria, AMR genes/gene combinations (especially plasmid borne genes), and AMR commensals – with particular reference to those raw materials which may not be subject to effective (lethal) antibacterial treatments prior to/during processing or prior to consumption.

81. As well as the above food specific knowledge gaps, other food processing related activities can, if incorrectly executed, encourage sublethal stress conditions (and more persistent mutants), in food environments. Thus, the formation and persistence of biofilms, and inadequate biocide/sanitiser based decontamination treatments are being increasingly recognised as AMR development “hot spots” (Lerma *et al.*, 2013, Molina-Gonzalez *et al.*, 2014, Gadea *et al.*, 2017, Pal, 2017).

82. **Priority rating: This area is probably of considerable interest, but less immediate specific priority, to FSA. Thus, biofilms and biocides within food processing environments are already known to share many characteristics and challenges with biofilms and biocides in inanimate clinical and environmental surfaces. However, increasing recognition of food related**

biofilms as significant AMR hot spots, with very undesirable implications, particularly in ready-to-eat (RTE) foods, suggests that FSA should consider an initial overview/review of the potential impacts of food processing surfaces biofilms in relation to ToR (i).

83. **Recommendation - FSA should work with other relevant government agencies in reducing the knowledge gaps associated with the AMR generation /dissemination impact of the formation and persistence of biofilm on food contact surfaces, with particular reference to inadequate biocide/sanitiser decontamination treatments/rotations in industrial, retail and domestic environments.**

Human food

Background

84. There is a consensus that food production animals constitute the main reservoir of organisms, both pathogenic and non-pathogenic, which possess antimicrobial drug resistance (AMR) genes which can be transmitted to humans by a variety of methods or routes (ECDC/EFSA/EMA, 2017a). Furthermore, foods are regarded as a principal intermediary for such genes. Nevertheless, there is a lack of comprehensive data on the involvement of foods, both of animal and non-animal origin as carriers of AMR genes, particularly in respect of those capable of mediating resistance to what are regarded as Critically Important Antimicrobials (CIAs) (WHO, 2017).

85. In respect of *E. coli* exhibiting resistance to extended spectrum β-lactamases (ESBLs) a recently-published study on the occurrence of ESBL- carrying *E. coli* (ESBL-EC) s in meat, animals, farm slurry and vegetables has demonstrated that 2 % of beef, 3% of pork and 65% of chicken samples were positive for ESBL-EC, whereas all fruit and vegetables tested were negative for ESBL-EC. None of the foodstuffs yielded *E. coli* with CTX-M-15 ESBL, which dominates in human clinical isolates in the UK (Randall *et al.*, 2017).

86. Although none of the isolates examined in the above study yielded carbapenem-resistant E. coli, there is increasing concern that foods imported into the UK, particularly from countries that are outside the EU and as such are not subject to current EU requirements regarding the use of antimicrobials in food-production animals, may harbour carbapenemase-producing bacteria originating from such animals.. This scenario may become a real threat if and when food imports from such countries increase after the implementation of EU exit.

87. **Areas which are important and which we are unaware of others doing work on at present. These are for the FSA to consider as priority.**

    - There is a lack of AMR prevalence data for British-produced food and, to a lesser extent in countries that export food to the UK, with a notable exception of certain major food exporting countries in northern Europe.
    - Information on AMR bacteria in food is not sufficiently comprehensive for meaningful conclusions on incidence to be made.
    - There is a lack of knowledge on the significance of the food chain in the context of AMR with particular reference to red meat, eggs and egg products (UK origin and imported) on retail sale in the UK.
    - Although plasmid-mediated resistance to colistin has been reported in both *Salmonella* spp. and *Escherichia coli* from pigs on a farm in Great Britain(Anjum *et al.*, 2016), there is a lack of comprehensive knowledge of the spread of such resistance in organisms from foods in the UK, both home-produced and imported.

- Consideration of a risk ranking of imported foods (both European and non-European) in relation to AMR.
- The influence of food production processes – e.g. carcass decontamination measures, storage, chilling, freezing, etc on AMR.

88. **Areas which we consider are important but not necessarily for FSA to address**

- Data on antimicrobial use (AMU) in food-producing animals at species level in the UK is important in seeking to explain the occurrence and dynamics of AMR, resistance genes and MDR phenotypes in a defined geographical area. More complete information should therefore be collected on the type of production systems from which food samples originate to assess the impact of animal husbandry practices as risk factors for resistance. This is a task that is primarily a responsibility of the Veterinary Medicines Directorate (VMD)
- There is a need for more studies to quantify the contribution of both domestic and imported foods to the occurrence of AMR in food consumed in the UK. Information on country of origin for imported products should therefore be collected and care should be taken to ensure that the country of origin of such food is clearly stated on packaging or such information can be obtained.

89. **Areas which we consider are of interest but of lower priority**

- Possible use of the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) when undertaking any further systematic reviews of AMR in humans, food-producing animals and foods thereof.

90. **Recommendations for FSA to consider**

- **High priority recommendation -** Further research and surveillance is needed to continue quantifying the risk of transmission to humans of antimicrobial resistance genes, and particularly those encoding resistance to Critically Important Antimicrobials (CIAs), including plasmid-mediated colistin resistance in organisms from foods of both animal and non-animal origin, both UK-produced and imported.
- **High priority recommendation** - Regular targeted surveillance of specific food items for AMR is essential, including both foods of animal and non-animal origin, and both home-produced and imported foods
- Research and surveillance efforts should be continued to monitor AMR trends in both foodborne pathogens and commensal bacteria in UK and imported chicken and poultry meat.
- Efforts should be made to systematically assess the influence of food production processes – e.g. carcass decontamination measures, storage, chilling, freezing, etc on AMR.
- Consideration of a risk ranking of imported foods (both European and non-European) in relation to AMR.

Humans

Background

91. At present, information on antimicrobial resistance in food is not sufficiently comprehensive to fully inform the study of, or to support conclusions around, transmission to or from humans. Initial research findings suggest a relatively minor role for food in the acquisition and spread of AMR in humans. Although current risk assessments e.g. LA-MRSA are based on best available evidence, it is recognised that there is a lack of data in this area, some of which is based on research carried out more than 10 years ago. There is considerable focus on AMR in the press, broadcast and social media, and other online sources. It is likely that this will continue to fuel understandable public concerns regarding the potential role of transmission from foodstuffs to humans. Given our current state of knowledge in this area, the formulation of advice for consumers will be reliant upon expert consensus opinion in the short to medium term.

92. **Areas of importance – sufficient/good research & no obvious need for more work**

- Previous national and international reports, such as "The Path of Least Resistance" (SMAC, 1998), the "UK 5 Year Antimicrobial Resistance Strategy 2013-2018" (DoHSC, 2013), the World Health Organisation's "Global Action Plan on Antimicrobial Resistance" (WHO, 2015) and The O'Neill Report "Tackling Drug-Resistant Infections Globally: Final Report and Recommendations" (O'Neill, 2016), provide a comprehensive assessment of the importance of antimicrobial resistance, clearly setting out the prevalence, mechanisms, routes of transmission and the likely impact on health as well as identifying potential risk management interventions.

93. **Areas of importance – unaware of others covering these areas; potential priority area for FSA; potential priority areas for agencies other than the FSA to address**

- **Priority recommendation**- Co-ordinated research. FSA should continue to monitor the relative importance of AMR in food relative to other contributory factors. There appears to be a significant gap in the overall governance of research activity into antimicrobial resistance and food. At present, most research outputs appear to result from the intermittent availability of largely uncoordinated, opportunistic funding. The FSA might consider working with others, nationally and internationally, both to help define the scope of research studies and ongoing surveillance that is required in the field of antimicrobial resistance and humans as well as contributing to coordinated funding mechanisms that might facilitate a more structured and effective research programme. ***(FSA) e.g. FSA should press at AMR FUNDERS FORUM the relative importance to continue to monitor all areas including food.***

- Recommendation - There are very limited data on the role of food in the acquisition & spread of antimicrobial resistance genes (as opposed to entire organisms) to humans. Concurrent, sequential application of WGS analysis of organisms in food and humans is likely to represent a major advance in addressing this gap. ***(FSA & Others)***

- **Priority recommendation** - Regular targeted surveillance of specific food items for AMR is essential although initial research findings suggest a relatively minor role for food in the acquisition and spread of AMR in humans. However, sequential studies and ongoing surveillance are needed to monitor changes in AMR patterns both nationally and internationally to help define the source/recipient direction of the spread of resistance and to assess the effectiveness of risk management interventions. ***(FSA & Others)***

- Recommendation - There is a need to ensure that appropriate isolates from surveys and surveillance of humans, animals, food and environment are either subject to WGS analysis to elucidate phylogenetic relationships and/or nucleic acid is retained from such samples to facilitate future analysis. ***(FSA & Others)***

94**. Areas of lower priority**

- There is probably a need to look at ways in which existing data sources/research, including "grey" literature resources, can be better captured and synthesised to inform interventions and policy. This will also include capturing inputs from a wide range of sources, including economic and social data, and extending beyond the conventional academic realm. It will require the use of emerging data mining and informatics approaches, combined with the increasing use of mathematical models. While this is unlikely to be an FSA priority *per se* in the current context, it is likely that these types of approaches will be important in the future to inform decisions in various areas of the Agency's activities. This will help to address some of the current limitations of the way scientific research reporting has traditionally been undertaken. An example of this approach is the way in which online databases of genomic data are mined by groups of researchers who did not necessarily generate the data in the first place. This is also much more efficient in terms of the use of available resources.

General conclusions and overarching themes identified by the group

During their discussions, the group identified overarching conclusions impacting on the development, dissemination and impact of antimicrobial resistance (AMR) in the food chain. These included:

- A significant lack of antimicrobial and AMR data in relation to UK produced or processed food, or food imported to the UK, in absolute and comparative terms. Much of the readily available UK AMR data have been obtained indirectly as a consequence of research focussing on themes other than AMR.

  More information is needed on factors which affect the dynamics of AMR gene transfer between commensals and pathogens. Although commensals can be a significant reservoir of AMR genes, comparatively little is known about such transfers in food and food processing activities.

- EU-exit- related changes in the relative amounts of foods imported from non-EU countries are likely to change the qualitative and quantitative antimicrobial and AMR- related challenges in foods consumed in the UK.

- Current hygiene standards applied across the food sector would benefit from being revisited to ensure that measures are in place to incorporate specifically AMR-related concerns. For example, an over-stringent approach to hygiene may have the effect of selecting more resilient AMR bacteria. Similarly, the inappropriate use of agents such as biocides in abattoirs and food processing environments may promote cross-selection of antimicrobial-resistant organisms.

- Consumer education programmes should be reviewed in response to increased risks associated with contact with raw pet foods, consumption of raw drinking milk, etc. to limit potential exposure to AMR bacteria. The monitoring of residues in milk should be continued and extended to detect the wider range of antimicrobial classes occurring in such products.

- The modes of action of alternatives to antimicrobials needs to be better explored and defined. Any possible effects relating to the use of these alternatives to antimicrobials in animal feed and any unintended effects relating to co-selection and transmission of AMR genes in animals as a result also needs to be explored.

- There needs to be co-ordinated, regular, targeted surveillance to identify the contribution that food makes to AMR in humans relative to other routes of exposure. This work needs to be joined up across all sectors in an ongoing effort using a "One Health" approach which considers that ultimately, acquisition of AMR in/from food may be linked to apparently separate activities for example manufacture, waste, disposal etc. Where grey areas are identified in terms of ownership for AMR-related work, there needs to be clarification.

- WGS data and appropriate bioinformatics pipelines have the potential in investigating and resolving significant gaps in the epidemiology and ecology of AMR at various stages of the human food chain.

- There are significant levels of uncertainty in many aspects of food/AMR interaction and consequently, a need for co-ordinated, targeted research to address these gaps.

- The group noted considerable industry emphasis on antimicrobial stewardship within food production as the primary intervention to control AMR. AMR stewardship is important and considerable progress is being made in this area, However, other aspects are also important. Thus, it remains important to consider and assess concurrent intervention strategies such as biosecurity, hygiene, infection control, animal & plant husbandry, and the role of alternative agents in the management and prevention of infection/cross contamination during food production (vaccination, disinfection etc), along with structured surveillance and appropriate producer & consumer education,

- Methods originally developed for the control of pathogens associated with foods are now being adapted and applied in an attempt to control AMR. While this appears to be a reasonable hypothesis, there is a need for planned evaluation to determine the actual efficacy of this approach.

Glossary

ACMSF - Advisory Committee on the Microbiological Safety of Food
AMR - antimicrobial resistance
ARG – Antimicrobial gene
BPC- British Poultry Council
CIAs- Critically Important Antibiotics
DEFRA- Department for Environment, Food and Rural Affairs
DoHSC – Department of Health and Social Care
ECDC- European Centre for Disease Prevention and Control
ECOFF- Epidemiological cut-off (resistance values)
EFSA- European Food Safety Authority
ESBL – Extended Spectrum Beta Lactamases
EU - European Commission
EMA – European Medicines Agency
FBs – Food Businesses
HPCIA - High Priority Critically Important Antibiotics
JIACRA - Joint Interagency Antimicrobial Consumption and Resistance Analysis Report
LA-MRSA- Livestock associated MRSA
MAP – Modified Atmosphere Packaging
MDR - Multiple Drug Resistance
OIE – World Organisation for Animal Health
(UK)VARSS-Veterinary Antimicrobial Resistance and Sales Surveillance
RMBD – Raw Meat Based Diets
RUMA - The Responsible use of Medicines in Agriculture Alliance
RTE – Ready to Eat
ToRs – (FSA) Terms of Reference
VMD – Veterinary Medicine Directorate
WGS - Whole Genome Sequencing
WHO – World Health Organisation

References

ACMSF (1999) 'Report on Microbial Antibiotic Resistance in Relation to Food Safety Stationary Office, London'.

Al-Nabulsi, A.A. *et al.* (2015) 'Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of Listeria monocytogenes'. *Food Microbiology,* 46 154-160.

Amachawadi, R.G. *et al.* (2015) 'Effects of In-feed copper, chlortetracycline, and tylosin on the prevalence of transferable copper resistance gene, tcrb, among fecal enterococci of weaned piglets'. *Foodborne Pathogens and Disease,* 12 (8), pp. 670-678.

Anjum, M.F. *et al.* (2016) 'Colistin resistance in Salmonella and Escherichia coli isolates from a pig farm in Great Britain'. *Journal of Antimicrobial Chemotherapy,* 71 (8), pp. 2306-2313.

Arnold, K.E., Williams, N.J. and Bennett, M. (2016) ''Disperse abroad in the land': the role of wildlife in the dissemination of antimicrobial resistance'. *Biology Letters,* 12 (8), pp.

Bangerter, P.D. *et al.* (2016) 'Longitudinal study on the colonisation and transmission of methicillin-resistant Staphylococcus aureus in pig farms'. *Veterinary Microbiology,* 183 125-134.

Beaber, J.W., Hochhut, B. and Waldor, M.K. (2004) 'SOS response promotes horizontal dissemination of antibiotic resistance genes'. *Nature,* 427 (6969), pp. 72-74.

Blau, K. *et al.* (2017) 'Soil texture-depending effects of doxycycline and streptomycin applied with manure on the bacterial community composition and resistome'. *FEMS microbiology ecology*.

Cahill, S.M. *et al.* (2017) 'Global Perspectives on Antimicrobial Resistance in the Food Chain. FAO'. *Food Protection Trends,* 37 (6), pp. 353 - 360.

Ceuppens, S. *et al.* (2014) 'Molecular methods in food safety microbiology: Interpretation and implications of nucleic acid detection'. *Comprehensive Reviews in Food Science and Food Safety,* 13 (4), pp. 551-577.

Chen, Q.-L. *et al.* (2017) 'Do manure-borne or indigenous soil microorganisms influence the spread of antibiotic resistance genes in manured soil?'. *Soil Biology & Biochemistry,* 114 229-237.

Chen, Q. *et al.* (2016) 'Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil'. *Environment International,* 92-93 1-10.

Cirz, R.T. and Romesberg, F.E. (2007) 'Controlling mutation: intervening in evolution as a therapeutic strategy'. *Critical Reviews in Biochemistry and Molecular Biology,* 42 (5), pp. 341-354.

Cohen, B.E. (2014) 'Functional linkage between genes that regulate osmotic stress responses and multidrug resistance transporters: challenges and opportunities for antibiotic discovery. '. *Antimicrobial Agents & Chemotherapy,* 58 640-646.

Cohen, N.R., Lobritz, M.A. and Collins, J.J. (2013) 'Microbial Persistence and the Road to Drug Resistance'. *Cell Host & Microbe,* 13 (6), pp. 632-642.

Cohen, N.R. *et al.* (2016) 'A role for the bacterial GATC methylome in antibiotic stress survival'. *Nature Genetics,* 48 (5), pp. 581-+.

Cook, K.L., Netthisinghe, A.M.P. and Gilfillen, R.A. (2014) 'Detection of pathogens, indicators, and antibiotic resistance genes after land application of poultry litter'. *Journal of Environmental Quality,* 43 (5), pp. 1546-1558.

DoHSC (2013) *UK 5 Year Antimicrobial Resistance Strategy 2013 to 2018* *https://www.gov.uk/government/publications/uk-5-year-antimicrobial-resistance-strategy-2013-to-2018*.

E-A (2017) *Environment Agency, Soil screening values for assessing ecological risk* *https://www.gov.uk/government/publications/soil-screening-values-for-assessing-ecological-risk*.

ECDC/EFSA/EMA (2017a) '(JIACRA) ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals: Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report'. *EFSA Journal,* 15 (7), pp.

ECDC/EFSA/EMA (2017b) *Joint Scientific Opinion on a list of outcome indicators as regards surveillance of antimicrobial resistance and antimicrobial consumption in humans and food-producing animals."* *http://ecdc.europa.eu/sites/portal/files/documents/AMR-indicators-joint-report-Oct-2017.pdf*

EFSA-BIOHAZ (2017) 'Risk for the development of antimicrobial resistance (AMR) due to feeding of calves with milk containing residues of antibiotics'. *EFSA Journal,* 15 (1), pp.

EFSA (2010) *Quantitative Microbiological Risk Assessment on Salmonella in Slaughter and Breeder pigs: Final Report.* *http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2010.EN-46/abstract*.

Fahrenfeld, N. *et al.* (2014) 'Effect of Manure Application on Abundance of Antibiotic Resistance Genes and Their Attenuation Rates in Soil: Field-Scale Mass Balance Approach'. *Environmental Science & Technology,* 48 (5), pp. 2643-2650.

Filippitzi, M.E. *et al.* (2016) 'Risk of cross-contamination due to the use of antimicrobial medicated feed throughout the trail of feed from the feed mill to the farm'. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment,* 33 (4), pp. 644-655.

Foster, P.L. (2007) 'Stress-Induced Mutagenesis in Bacteria'. *Critical Reviews in Biochemistry and Molecular Biology,* 42 373-397.

Fredriksson-Ahomaa, M. *et al.* (2017) 'Raw meat-based diets in dogs and cats'. *Veterinary sciences,* 4 (3), pp.

Fruci, M. and Poole, K. (2016) 'Bacterial Stress Responses as Determinants of Antimicrobial Resistance *'*. In*:* FJd, B. (ed.) *Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria,.* John Wiley, pp. 115 - 136.

Gadde, U. *et al.* (2017) 'Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review'. *Animal Health Research Reviews,* 18 (1), pp. 26-45.

Gadea, R. *et al.* (2017) 'Effects of exposure to quaternary-ammonium-based biocides on antimicrobial susceptibility and tolerance to physical stresses in bacteria from organic foods'. *Food Microbiology,* 63 58-71.

Gryson, N. (2010) 'Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review'. *Analytical and Bioanalytical Chemistry,* 396 (6), pp. 2003-2022.

Harms, A., Maisonneuve, E. and Gerdes, K. (2016) 'Mechanisms of bacterial persistence during stress and antibiotic exposure'. *Science,* 354 (6318), pp.

Hodgson, C.J. *et al.* (2016) 'Seasonal persistence of faecal indicator organisms in soil following dairy slurry application to land by surface broadcasting and shallow injection'. *Journal of Environmental Management,* 183 325-332.

Lau, C.H.-F. *et al.* (2017) 'Impact of pre-application treatment on municipal sludge composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest'. *Science of the Total Environment,* 587 214-222.

Leclercq, S.O. *et al.* (2016) 'A multiplayer game: species of Clostridium, Acinetobacter, and Pseudomonas are responsible for the persistence of antibiotic resistance genes in manure-treated soils'. *Environmental Microbiology,* 18 (10), pp. 3494-3508.

Lerma, L.L. *et al.* (2013) 'Prevalence of bacteria resistant to antibiotics and/or biocides on meat processing plant surfaces throughout meat chain production'. *International Journal of Food Microbiology,* 161 (2), pp. 97-106.

Marti, R. *et al.* (2013) 'Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest'. *Applied and Environmental Microbiology,* 79 (18), pp. 5701-5709.

Marti, R. *et al.* (2014) 'Safely Coupling Livestock and Crop Production Systems: How Rapidly Do Antibiotic Resistance Genes Dissipate in Soil following a Commercial Application of Swine or Dairy Manure?'. *Applied and Environmental Microbiology,* 80 (10), pp. 3258-3265.

Mateus, A. *et al.* (2016) 'A systematic review to assess the significance of the food chain in the context of antimicrobial resistance (AMR) with particular reference to pork and poultry meat, dairy products, seafood and fresh produce on retail sale in the UK.'.

McKellar, R.C. *et al.* (2014) 'Evaluation of different approaches for modeling Escherichia coli O157:H7 survival on field lettuce'. *International Journal of Food Microbiology,* 184 74-85.

McMahon, M.A.S. *et al.* (2007a) 'The rate of horizontal transmission of antibiotic resistant plasmids is increased in food preservation-stressed bacteria'. *International Journal of Antimicrobial Agents,* 29 S612-S612.

McMahon, M.A.S. *et al.* (2007b) 'Environmental stress and antibiotic resistance in food-related pathogens'. *Applied and Environmental Microbiology,* 73 (1), pp. 211-217.

Molina-Gonzalez, D. *et al.* (2014) 'Effect of sub-lethal concentrations of biocides on the susceptibility to antibiotics of multi-drug resistant Salmonella enterica strains'. *Food Control,* 40 329-334.

Moon, D.C. *et al.* (2015) 'Identification of livestock-associated methicillin-resistant Staphylococcus aureus isolates in Korea and molecular comparison between isolates from animal carcasses and slaughterhouse workers'. *Foodborne Pathogens and Disease,* 12 (4), pp. 327-334.

Muurinen, J. *et al.* (2017) 'Influence of manure application on the environmental resistome under finnish agricultural practice with restricted antibiotic use'. *Environmental Science & Technology,* 51 (11), pp. 5989-5999.

Nair, C.G. *et al.* (2013) 'Sub-lethal concentrations of antibiotics increase mutation frequency in the cystic fibrosis pathogen Pseudomonas aeruginosa'. *Letters in Applied Microbiology,* 56 (2), pp. 149-154.

Normanno, G. *et al.* (2015) 'Methicillin-resistant Staphylococcus aureus (MRSA) in slaughtered pigs and abattoir workers in Italy'. *Food Microbiology,* 51 51-56.

O'Neill (2016) 'Tackling drug-resistant infections globally: Final report and recommendations. https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf'.

Ongeng, D. *et al.* (2014) 'Modeling the fate of Escherichia coli O157:H7 and Salmonella enterica in the agricultural environment: Current perspective'. *Journal of Food Science,* 79 (4), pp. R421-R427.

Pal, C. (2017) *Effects of biocides and metals on antibiotic resistance: a genomic and metagenomic perspective.* PhD Gothenburg.

Poole, K. (2012) 'Stress responses as determinants of antimicrobial resistance in Gram-negative bacteria'. *Trends Microbiol,* 20 (5), pp. 227-234.

Randall, L.P. *et al.* (2017) 'Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant Escherichia coli'. *International Journal of Food Microbiology,* 241 283-290.

RUMA (2017) *RUMA task force new antibiotic usage targets* http://www.thepigsite.com/swinenews/44323/ruma-task-force-announces-new-antibiotic-usage-targets/.

SCENIHR (2009) 'Assessment of the Antibiotic Resistance Effects of Biocides. Scientific Committee on Emerging and Newly Identified Health Risks, European Commission: Brussels, Belgium,'.

SMAC (1998) *Standing Medical Advisory Committee The Path of Least Resistance* http://antibiotic-action.com/wp-content/uploads/2011/07/Standing-Medical-Advisory-Committee-The-path-of-least-resistance-1998.pdf.

Song, J. *et al.* (2017) 'Comparison of metals and tetracycline as selective agents for development of tetracycline resistant bacterial communities in agricultural soil'. *Environmental Science & Technology,* 51 (5), pp. 3040-3047.

Stecher, B. *et al.* (2012) 'Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae'. *Proceedings of the National Academy of Sciences of the United States of America,* 109 (4), pp. 1269-1274.

Tezel, U. and Pavlostathis, S.G. (2011) 'Role of quaternary ammonium compounds on antimicrobial resistance in the environment.'. In: Keen, P.L. and Montforts, M.H.M.M. (eds.) *Antimicrobial resistance in the environment.* Hoboken, NJ.: John Wiley & Sons, Inc.,, pp. 349-387.

Tien, Y.-C. *et al.* (2017) 'Impact of dairy manure pre-application treatment on manure composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest'. *Science of the Total Environment,* 581 32-39.

Trevisani, M. *et al.* (2017) 'Effects of sanitizing treatments with atmospheric cold plasma, SDS and lactic acid on verotoxin-producing Escherichia coli and Listeria monocytogenes in red chicory (radicchio)'. *Food Control,* 78 138-143.

UK-VARSS (2016) 'Veterinary Antimicrobial Resistance and Sales Surveillance 2015 https://www.gov.uk/government/publications/veterinary-antimicrobial-resistance-and-sales-surveillance-2015'.

Vahjen, W. *et al.* (2015) 'High dietary zinc supplementation increases the occurrence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs'. *Gut Pathogens,* 7.

Van Meervenne, E. *et al.* (2015) 'Low temperature and modified atmosphere: Hurdles for antibiotic resistance transfer?'. *Journal of Food Protection,* 78 (12), pp. 2191-2199.

Wales, A. and Davies, R. (2015) 'Co-Selection of Resistance to Antibiotics, Biocides and Heavy Metals, and Its Relevance to Foodborne Pathogens'. *Antibiotics,* 4 (4), pp. 567.

Wang, F.-H. *et al.* (2015) 'Antibiotic resistance genes in manure-amended soil and vegetables at harvest'. *Journal of Hazardous Materials,* 299 215-221.

Wang, S. *et al.* (2016) 'Antimicrobial Peptides as Potential Alternatives to Antibiotics in Food Animal Industry'. *International Journal of Molecular Sciences,* 17 (5), pp.

WHO (2015) *Global action plan on antimicrobial resistance* http://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/.

WHO (2016) *At UN, global leaders commit to action on antimicrobial resistance. Press Release. Available at: http://www.who.int/mediacentre/news/releases/2016/commitment-antimicrobial-resistance/en/. Accessed 26 Feburary 2018.*

WHO (2017) *Global priority list of antibiotic-resistant bacteria to guide research, discovery and development of new antibiotics. http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/.*

Woolhouse, M. *et al.* (2015) '*Antimicrobial resistance in humans, livestock and the wider environment*'. *Philosophical Transactions of the Royal Society B-Biological Sciences.*

Zhang, X. *et al.* (2000) 'Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice'. *Journal of Infectious Diseases,* 181 (2), pp. 664-670.

Zhang, X. *et al.* (2014) 'Recent progress on atmospheric and room temperature plasma mutation breeding technology and its applications'. *CIESC Journal,* 65 (7), pp. 2676-2684.